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Effect of Local Injections of Penicillin on Staphylococci in the Cow's Udder

LOUIS A. KLEIN, V.M.D., DAVID W. CRISMAN, B.S., D.V.M., and JOHN W. MOOR, V.M.D.
Philadelphia, Pennsylvania

THE RESULTS obtained with local injections of penicillin in the treatment of empyema, mastoiditis, and chronic wound sinuses in man due to staphylococcus or streptococcus infection, as reported by the Floreys,[1] the National Research Council's Committee on Chemotherapeutic and Other Agents,[2] and by Lyons,[3] suggest that intramammary injections *via* the teat canal might be effective in catarrhal mastitis, also called chronic mastitis.

In this form of mastitis, conditions exist that have been found favorable to the action of local injections in treating human patients, *viz.*: The infection is situated in a closed cavity or space from which the contents can be readily withdrawn, and the infection is localized and accessible. Frei[4] and other investigators have found that in catarrhal mastitis due to streptococci, it is the excretory channels that are involved, usually the milk cistern and the larger ducts, primarily, while clinical symptoms indicate that the disease is similarly situated when it is caused by staphylococci. In this location the causative organisms are readily accessible to solutions introduced through the teat canal while any exudate present can be readily milked out before making an injection. Most clinicians with experience in treating human patients with the drug are of the opinion that local injections should be supplemented by intravenous or intramuscular injections but recovery or improvement has been obtained in the conditions mentioned above with local injections alone.

It was decided to test the action of intramammary injections of penicillin on the staphylococci which cause mastitis and also to determine the effect on the cow, especially on the udder tissue. The penicillin used was prepared specifically for this study by Dr. Walter Kocholaty, Thos. H. Dougherty Jr. Fellow in Research in Brucellosis, University of Pennsylvania School of Medicine, in July, 1943.

Staphylococci are one of the causes of catarrhal mastitis and the symptoms they produce are similar to those caused by streptococci. They also cause a severe type of acute parenchymatous mastitis which is often complicated by sepsis, and gangrene of the udder is frequently associated with it. This type of udder infection was selected because clinical results show that it is more resistant to the agents now used in intramammary injections than the mastitis streptococci, and also because in *in vitro* tests penicillin was found to be more effective than some of these agents against

From the School of Veterinary Medicine, University of Pennsylvania, Philadelphia.

In accordance with regulations formulated by the War Production Board, concerning the production and use of penicillin, permission was requested and received by Dr. G. A. Dick, Dean of the School of Veterinary Medicine, to produce and use a limited amount of penicillin for the purpose of this study. At the request of Dr. A. N. Richards, vice president of the University of Pennsylvania and chairman of the Committee on Medical Research of the Office of Scientific Research and Development, publication of the findings of this study has been withheld until the present. The contents of this paper were, however, submitted in confidence on Apr. 29, 1944, to the Bureau of Animal Industry, U. S. Department of Agriculture, and to the Pennsylvania Department of Agriculture.

Publication of this article has been cleared through the Committee on Medical Research, National Research Council.

This study was facilitated by laboratories of the University of Pennsylvania, which receive financial support from the U. S. Department of Agriculture, the Department of Agriculture of the Commonwealth of Pennsylvania, the Smith, Kline and French Laboratories Inc., and the Thos. H. Dougherty Jr. Fund.

Staphylococcus aureus. Fleming[5] found it four times as potent as sulfathiazole and twenty times as potent as sulfapyridine against this organism, and Heilman and Herrell[6] found it more effective than gramicidin.

IDENTIFICATION OF MASTITIS STAPHYLOCOCCI

In examining samples of milk or other udder secretions for the staphylococci which cause mastitis, it is necessary to differentiate them from the nonpathogenic cocci, usually called micrococci, which are normally present in the udder and the milk.

Minett[7] found that hemolysis on ox-blood or sheep-blood agar and coagulation of human or rabbit-blood plasma were characteristic of staphylococci isolated from milk samples, from udder quarters affected with chronic mastitis, and from the affected quarters of cows showing symptoms of acute, septic parenchymatous mastitis, and that it is improbable that cocci recovered from samples of milk from normal quarters, which do not possess these properties, are pathogenic. Plastridge and his associates[8] examined staphylococci isolated from milk samples from individual quarters of milking cows for their ability to hemolyze ox blood and coagulate human-blood plasma, for pigment formation, leucocyte count, and other properties, using the leucocyte count as an index of pathogenicity. From the results obtained, they concluded that ability to coagulate rabbit or human-blood plasma is more closely related to pathogenicity than any other property. The greater number of the coagulase positive strains were hemolytic but some were not. They also concluded that staphylococci from the udder which are hemolytic on ox-blood agar are usually pathogenic whether they have coagulative ability or not, as are also staphylococci associated with a leucocyte count of 500,000 or over provided the cow is not in the early or late stage of lactation. They found no definite correlation between pigment formation and the leucocyte count. Nearly as many strains of staphylococci associated with a low leucocyte count produced pigmented colonies as those associated with a high count. Chapman, Berens, Peters, and Curcio,[9] who studied staphylococci of human origin and determined pathogenicity by injecting cultures into rabbits, found that while strains which coagulated human-blood plasma were usually pathogenic regardless of the color of the colonies, those which produced hemolytic yellow colonies were usually pathogenic regardless of coagulative ability and hemolytic noncoagulating strains that produced white colonies were usually nonpathogenic.

TABLE 1 — Schedule Followed in Taking Milk Samples and Making Injections of Penicillin

TIME	QUARTERS RECEIVED 8 INJECTIONS AT 6-HOUR INTERVALS	QUARTERS RECEIVED 4 INJECTIONS AT 12-HOUR INTERVALS
First day:	Took Sample 1	Took Sample 1
3 p.m.	Milked out and injected quarter	Milked out and injected quarter
9 p.m.	Milked out and injected quarter	
Second day:	Took Sample 2	Took Sample 2
3 a.m.	Milked out and injected quarter	Milked out and injected quarter
9 a.m.	Milked out and injected quarter	
3 p.m	Took Sample 3	Took Sample 3
	Milked out and injected quarter	Milked out and injected quarter
9 p.m.	Milked out and injected quarter	
Third day:	Took Sample 4	Took Sample 4
3 a.m.	Milked out and injected quarter	Milked out and injected quarter
9 a.m.	Milked out and injected quarter	
3 p.m.	Took Sample 5	Took Sample 5

Took Samples 6, 7, 8, and 9 on the third, sixth, tenth, and thirteenth days following, immediately before the evening milking.

PLAN OF EXPERIMENT

In selecting the cows for this experiment, ability to hemolyze ox blood, coagulate human-blood plasma, and produce pigment were used, together with the leucocyte count, to differentiate pathogenic staphylococci from the nonpathogenic cocci (micrococci) which are com-

TABLE 2 — Results of Examination of Milk Samples Collected Immediately Before the First Injection of Penicillin

Cow	Quarter	Staphylococci per cc.	Pigmentation	Hemolysis	Coagulation	Leucocytes per cc.
91	LH	80	Medium	Hemolytic	—	1,200,000
10	LF	125	Orange	Hemolytic	+	60,000
10	RH	5400	Light	Hemolytic	+	900,000
1	RH	470	Light	Nonhemolytic	+	1,200,000
27	RF	175	Medium	Hemolytic	+	120,000
216	RF	510	Medium	Hemolytic	—	90,000
10	RF	5100	Light	Hemolytic	+	300,000

monly present in cow's milk. The methods by which these determinations were made are described below.

Samples of milk were taken from each quarter of the udder at the time of the evening milking but before the cow was milked. After the udder had been prepared in the usual way to prevent external contamination, three streams were expressed from the quarter and discarded, and 15 to 20 cc. of milk were then drawn into a sterile container, which was placed in a refrigerator until removed to the laboratory.

Plating.—Blood-agar plates were prepared by adding 5.0 per cent of defibrinated cow's blood to melted standard nutrient agar containing 0.5 per cent of sodium chloride and inoculating two plates from each milk sample, one with 1.0 cc. and one with 0.1 cc. After forty-eight hours incubation at 37 C., the plates were examined for hemolytic colonies and for nonhemolytic colonies showing pigmentation. Two plates of standard nutrient agar were inoculated with the same quantities of each sample and examined after forty-eight hours incubation, since pigmentation could be more readily detected on this medium. Hemolytic colonies of staphylococci and those showing any degree of pigmentation were picked off and streaked on standard nutrient agar. A film was also prepared from one of these colonies and gram-stained for microscopic examination. After the ox-blood agar and standard nutrient-agar plates were prepared, each milk sample was incubated for twenty-four hours and then streaked on ox-blood agar. These plates were examined after forty-eight hours incubation at 37 C.

Coagulase Test. — The technique used by Chapman, Berens, Peters, and Curcio,[2] as modified by Plastridge and associates,[3] was used to determine ability to coagulate human-blood plasma. (Rabbit-blood plasma is equally satisfactory.) A 4 mm. loopful of a 24-hour-old culture on nutrient agar was mixed with 0.5 cc. of fresh plasma contained in a 3/8 in. by 3 in. test tube, held at room temperature, and examined after one, two, three, and twenty-four hours, for evidence of clot formation. Evidence of clotting during the period of observation is a positive result. The human-blood plasma was obtained by drawing 75 cc. of blood in a flask containing 7.5 cc. of a 10 per cent solution of sodium citrate, and centrifuging to separate the plasma from the blood cells. The plasma should be used within forty-eight hours.

Leucocyte Count.—The slides for the leucocyte count were prepared and the count determined as specified for the direct microscopic

TABLE 3 — Results of Examination for Staphylococci of Milk Samples from Udder Quarters Receiving Eight Injections at Intervals of Six Hours

	Cow 91 L.H. Quarter			Cow 10 L.F. Quarter			Cow 10 R.H. Quarter			Cow 1 R.H. Quarter		
	Staphylococci			Staphylococci			Staphylococci			Staphylococci		
Sample	Poured plate	Streaked plate	Coagulation	Poured plate	Streaked plate	Coagulation	Poured plate	Streaked plate	Coagulation	Poured plate	Streaked plate	Coagulation
Before injection:												
1	H	H	—	H	H	+	H	H	+	NH	NH	+
After injection:												
2	—	—		—	—		—	—		—	—	
3	—	—		—	—		—	—		—	—	
4	—	—		—	—		—	—		—	—	
5	—	—		—	—		—	—		—	—	
6	—	—		—	—		H	H	+	NH	NH	—
7	—	—		—	H	+	H	H	+	—	NH	—
8	—	—		—	NH	—	H	H	+	—	NH	—
9	—	—		—	NH	—	H	H	+	—	NH	—

Ox-blood agar plates: H = hemolytic colonies; NH = nonhemolytic colonies; — = no staphylococci colonies; + = coagulation; — = no coagulation.

method of counting bacteria in milk in Standard Methods for the Examination of Dairy Products, 8th edition, pp. 44 to 54.

On the information obtained by the methods of examination described previously, seven quarters were selected for treatment with penicillin—three in 1 cow (10) and one each in 4 others (91, 1, 27, and 216). None of the cows showed any clinical symptoms of mastitis. According to the history obtainable, all had previously had attacks of catarrhal mastitis due to streptococci but none recently. On palpation of the selected quarters, indurated areas were found in four, viz: a 2 in. by 4 in. area in the left front quarter of cow 10 and a similar area in the right hind quarter; a nodular area 2½ in. in diameter in the right hind quarter of cow 1, with thickening of a part of the mucous membrane of the milk cistern, and an area 2½ in. in diameter in the right front quarter of cow 27.

SCHEDULE OF DOSAGE AND SAMPLING

Penicillin was used in the form of the sodium salt. This material, at the time of its preparation, assayed 320 Oxford units per milligram. It was contained in sealed glass vials, 120 mg. in each vial, and the vials were closed with rubber stoppers. Difficulty was encountered in making other arrangements for the study so the penicillin was stored in a refrigerator until its use nine months later, at which time, the assay value was 190 Oxford units per milligram. The contents of a single vial was dissolved in 500 cc. of sterile normal saline solution and injected in 1 dose. Each dose, therefore, contained 22,800 Oxford units of penicillin. The solution was prepared immediately before injection, the vials being stored in a refrigerator until that time.

Four of the quarters selected for treatment were given 8 injections of 500 cc. of the solution *via* the teat canal, at intervals of six hours, and three received 4 injections, at twelve-hour intervals. The treatment was begun at the time of the regular evening milking. Just before the cow was milked, a sample of milk was taken in the manner described previously, from the quarter to be injected. After the cow was milked by the regular milker, the quarter was stripped; the teat wiped with 70 per cent alcohol; and the first injection made. These samples were given the designation 1, and the containers were marked with this number and the number of the cow. The quarter was also indicated. Before each subsequent injection, the quarter to be injected was milked out as thoroughly as possible. Samples were taken every twelve hours from both groups of quarters, *i.e.*, from those receiving 8 injections and those receiving 4 injections. All cows in the experiment were milked before any of the other cows in the herd.

The schedule followed in making the injections and taking the milk samples for laboratory examination is presented in table 1. Referring to this table, it will be seen that the samples taken from the quarters receiving 8 injections and numbered 2, 3, 4, and 5 were drawn from the treated quarters six hours after the last of 2 injections, given six hours apart, and that the samples from the cows receiving four injections and numbered 2, 3, 4, and 5 were each taken twelve hours after a single injection. Samples were also taken from both groups on the third, sixth, tenth, and thirteenth days following the last injection and numbered 6, 7, 8, and 9, respectively. These samples were taken immediately before the evening milking. Each sample container was marked to indicate the number of the sample, the number of the cow, and the quarter.

The results of the examination of samples 1, which were taken immediately before the first injection, are shown in table 2. In samples taken previously from the left hind quarter of cow 91 and from the right front quarter of cow 27, the plate count of staphylococci was much higher than in samples 1, being 11,000 and 14,000 per cc., respectively. A great varia-

TABLE 4 — Results of Examination for Staphylococci of Milk Samples from Udder Quarters Receiving Four Injections

Sample	Cow 27 R.F. Quarter			Cow 216 R.F. Quarter			Cow 10 R.F. Quarter		
	Staphylococci			Staphylococci			Staphylococci		
	Poured Plates	Streaked Plates	Coagulation	Poured Plates	Streaked Plates	Coagulation	Poured Plates	Streaked Plates	Coagulation
Before injection:									
1	H	H	+	H	H	—	H	H	+
After injection:									
2	—	—		—	—		—	—	
3	—	—		—	—		—	—	
4	—	—		—	—		—	—	
5	—	—		—	—		—	—	
6	—	H	+	—	—		—	NH	+
7	—	H	+	—	H	—	—	H	+
8	—	H	+	H	H	—	—	NH	—
9	—	H	+	—	—		—	H	+

Ox-blood agar plates: H = hemolytic colonies; NH = nonhemolytic colonies; — = no staphylococci colonies; + = coagulation, — = no coagulation.

tion in the number of staphylococci in samples from the same source, even when they are collected at short intervals, has been reported by a number of investigators.

DISCUSSION OF RESULTS

The results of the examination for staphylococci of the samples collected after the injection of penicillin was begun and in the period following the last injection from the quarters receiving 8 injections are given in table 3. Similar information regarding the samples from the quarters receiving 4 injections is presented in table 4. On examination of these tables, it will be seen that staphylococci did not appear on any of the plates inoculated with the milk samples numbered 2, 3, 4, and 5, from either group. Those from the quarters included in table 3 were taken after 2 injections of penicillin had been given, the last six hours before, while those in table 4 had received 1 injection twelve hours previously. These results indicate that the antibacterial action of the penicillin continued for twelve hours and that up to this stage of the experiment, 4 injections at twelve-hour intervals were as effective as 8 at six-hour intervals.

The treated quarter of cow 91 remained free of the infection during the entire period the examination of milk samples was continued, *i.e.*, for thirteen days after the last injection. It is probable that the quarter treated in cow 1 also remained free of the original infection since the nonhemolytic strain of staphylococci in samples 6, 7, 8, and 9 from this quarter did not coagulate human-blood plasma as did the nonhemolytic strain which infected this quarter prior to the injection of penicillin. Both of these quarters received 8 injections at intervals of six hours. The two other quarters given the same treatment and also the three that received 4 injections at intervals of twelve hours were apparently free of the infection when samples 5 were taken six and twelve hours after the last injection, but samples 6 or 7, taken on the third and sixth day following the last injection and samples taken subsequently were found to be infected. Since one of the quarters receiving 8 injections was apparently cleared of the infection and another was probably cleared, while the infection disappeared only temporarily from the quarters receiving 4 injections, it would appear that a more prolonged antibacterial action was produced by 8 injections than by 4, although given within the same period of time. However, since no growth of staphylococci occurred on any of the plates inoculated with samples 2 to 5, inclusive, it is possible that they contained sufficient penicillin to inhibit growth of the organism and that growth occurred on the plates inoculated with samples taken subsequently because all of the penicillin injected had been absorbed or eliminated.

Referring to table 4, it will be noted that sample 6 from the right front quarter of cow 10 was infected with coagulase positive nonhemolytic staphylococci but that the staphylococci found in samples 7 and 9 were hemolytic. A similar variation was observed in milk samples examined before the injections were made. Coagulase positive nonhemolytic staphylococci were present in a sample taken from this quarter twelve days before sample 1.

The first 1/4 to 1/2 of the milk obtained from three of the quarters receiving injections at six-hour intervals contained slugs or strings of mucus and was watery after the first injection and the milk of the other quarter receiving the same treatment was similarly affected after the second injection. Later, this portion of the milking was of a greenish or yellowish color. This condition continued up to the time of taking samples 5, six hours after the last injection.

Similar changes occurred in the milk of the quarters receiving 4 injections at eight-hour intervals but to a lesser degree. The milk of one quarter in this group was altered in appearance after the first injection and of the other two after the second injection. The milk of one of the latter was of normal appearance at all other succeeding examinations.

When samples 6 were taken on the third day following the last injection, the milk of all quarters was of normal appearance.

The average daily milk production for one week before and for the first, second,

Cow	Before Treatment	1st Week After	2nd Week After	3rd Week After	Stage of Lactation when Treated
91	17.15 lb.	16.60 lb.	16.80 lb.	16.10 lb.	50th week
10	23.12 lb.	21.44 lb.	20.03 lb.	19.70 lb.	38th week
1	31.94 lb.	29.10 lb.	29.90 lb.	29.90 lb.	15th week
27	32.94 lb.	35.02 lb.	28.80 lb.	32.30 lb.	11th week
216	41.62 lb.	38.41 lb.	28.80 lb.	32.00 lb.	14th week

and third weeks after treatment are shown in the following table.

Cow 10 had three quarters injected, two with 8 doses and one with 4 doses. One quarter was treated in each of the others. Cows 91 and 1 received 8 doses and 27 and 216, 4 doses.

As to the effect of the injections on the udder tissues, only 1 cow, 91, showed any symptoms of irritation. The lower portion of the quarter treated in this cow was swollen and hardened three hours after the first injection but the swelling was not hot or painful to pressure. In a few hours, the entire quarter was involved and after the second injection the swelling increased slightly. No further change occurred until after the fourth injection. The swelling had begun to decrease and had disappeared entirely on the third day following the last dose.

The temperature of the cows was taken at the time of each injection and was within the normal range in all instances except at the time of the second injection, when it was 103.2 F. in cow 91, 103.2 F. in cow 10, and 103.6 F. in cow 27, and at the time of the third injection 102 F. in cow 216.

Summary

Seven udder quarters, infected with staphylococci, with characteristics corresponding to those of mastitis staphylococci, were given injections *via* the teat canal of a solution of penicillin in sterile, normal saline solution. Four quarters were given 8 injections of 500 cc. each at intervals of six hours and 3 received 4 injections at intervals of twelve hours. Each dose contained 22,800 Oxford units of penicillin.

Samples of milk were taken for laboratory examination immediately before the first injection, at intervals of twelve hours during the injection period, and on the third, sixth, tenth, and thirteenth days following.

Laboratory examinations indicated that the samples from all of the quarters taken after the first injection were free of the staphylococcus infection. The samples taken subsequently, up to six and twelve hours after the last injection, were also free, but the infection again developed on the plates inoculated with samples taken from five quarters on the third or sixth day after the last injection. The original staphylococcus infection had apparently been destroyed in the two other quarters. Both of these quarters received 8 injections.

The results indicate that the antibacterial action continued for twelve hours but 4 doses were not sufficient to obtain a permanent effect.

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In Vitro Studies of the Basis for Sulfanilamide Therapy in Bovine Mastitis

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THE SUBJECT of sulfanilamide therapy in bovine mastitis has received considerable attention in recent years. Although the early reports show contradictory results, more recent controlled studies have revealed that sulfanilamide administered orally to a cow is of little or no curative value in permanently eradicating the streptococci from the udders of cows suffering from acute or chronic mastitis. A new approach to the problem of sulfanilamide therapy in bovine mastitis has been made in this laboratory, and the results of the findings have been reported.[1] It was shown that where sulfanilamide is introduced in large amounts directly into the udder *via* the teat duct, mastitis streptococci are destroyed in most cases. The purpose of this report is to present a study of some of the fundamental principles which govern the activity of sulfanilamide in respect to the infecting organism.

CULTURES AND MEDIUMS

The strains of *Streptococcus agalactiae* employed in this study, with the exception of three cultures, had been isolated in this laboratory from chronic cases of mastitis. Cultures 090R, V-8 and K-151A were supplied by the Lederle Laboratories and represent, respectively, types I, II, and III of Lancefield group B. The identity of the cultures as *Str. agalactiae* was established by means of physiologic tests and serologic reactions, using the precipitin method of Lancefield and the rapid agglutination test. Some of the cultures had been carried on stock culture medium for some time previous to this study, while others were of recent isolation. The test organisms were transferred every twenty-four hours for two to three days to insure a rapid growth. Each culture was then diluted in sterile water blanks sufficiently so that 1 cc. contained approximately 500 to 2,000 living organisms. As will be shown later in this paper, the number of organisms present in the inoculum influences the degree and rapidity of their destruction by the sulfanilamide.

The mediums employed in these studies were tryptose broth and tryptose agar. Their composition was basically the same as described in a previous publication.[1]

Effect of Temperature on Bactericidal Activity of Sulfanilamide.—White and Parker[2] demonstrated that sulfanilamide at 20 mg. per cent is germicidal against group A streptococci at 40 C., but not at 37 C. Heishman and Miller[3] also reported that an increase in incubation temperature enhances the bactericidal action of sulfanilamide against group B streptococci. In view of the above reports, the following experiments were conducted to ascertain by quantitative determinations the effect of sulfanilamide on *Streptococcus agalactiae* at different temperatures. The tests were conducted as follows: 20 cc. of tryptose broth containing 20 mg. per cent of sulfanilamide were placed in 50 cc. flasks. The control flasks contained tryptose broth only. The test organisms were grown in tryptose broth for twenty-four hours and then diluted so that 1 cc. contained approximately 500 to 2,000 viable organisms. One cc. of the diluted culture was placed in each flask. All tests were run in duplicate; one set was incubated at 37 C. and the companion set was incubated at 40.5 C. At intervals of from five to twelve hours, a small portion was withdrawn from each flask and placed in serial dilutions to determine the number of living organisms at each interval. With some of the cultures the experiment was repeated with a variation in the inoculum which consisted of over one million organisms.

In chart 1, the results on four cultures are presented. The ordinates represent the number of living organisms at different intervals plotted in logarithmic scale, and the abscissas

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TABLE I.—Bactericidal Properties of Sulfanilamide on Streptococcus agalactiae Determined by the Cup-Plate Test

Hrs. Incubation of Petri dish with Sulfanilamide before Subculturing	Streptococcus agalactiae Culture																	
	1A				1B				1C				2A				3B				3C			
	No. Bacteria per Plate				No. Bacteria per Plate				No. Bacteria per Plate				No. Bacteria per Plate				No. Bacteria per Plate				No. Bacteria per Plate			
	438		4380		141		1410		400		4000		80		800		251		2510		80		800	
	a*	b**	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
24			+	+			+	+			+	+			+	+	+		+	+		+	+	+
48							+	+			+	+			+	+			+	+				
72							+	+			+	+			+	+			+	+				
96							+	+			+	+			+	+			+	+				
120							+	+			+	+			+	+			+	+				
Inhibition Zone	20 mm.		18 mm.		12 mm.		6 mm.		15 mm.		8 mm.		15 mm.		10 mm.		15 mm.		5 mm.		20 mm.		15 mm.	

Hrs. Incubation of Petri dish with Sulfanilamide before Subculturing	Streptococcus agalactiae Culture																	
	4A				4B				090R				V-8				K-151A			
	No. Bacteria per Plate				No. Bacteria per Plate				No. Bacteria per Plate				No. Bacteria per Plate				No. Bacteria per Plate			
	575		5750		430		4300		560		5600		144		1440		106		1060	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
24							+	+											+	+
48																				
72																				
96																				
120																				
Inhibition Zone	20 mm.		15 mm.		18 mm.		12 mm.		28 mm.		25 mm.		20 mm.		20 mm.		25 mm.		18 mm.	

Hrs. Incubation of Petri dish with Sulfanilamide before Subculturing	Streptococcus agalactiae Culture			
	F-12a-4			
	No. Bacteria per Plate			
	348		3480	
	a	b	a	b
24			+	+
48				
72				
96				
120				
Inhibition Zone	25 mm.		16 mm.	

*a = Results of subcultures made from Cup-Plate at 10 mm. from edge of cup.
**b = Results of subcultures made from Cup-Plate at 30 mm. from edge of cup.
+ = Viable organisms present.
− = No viable organisms.

represent the time intervals in hours. The results indicate that the organisms in the flasks which were incubated at 37 C. grew at approximately the same rate in both the control and the sulfanilamide flasks. At 40.5 C. the rate of growth was first retarded in both flasks, but soon a divergence occurred whereby the organisms in the control flask continued to grow until the maximum growth was reached, which was approximately the same as that in the 37 C. flasks; however, the organisms in the flasks with the 20 mg. per cent sulfanilamide were rapidly decreasing in number until all the organisms were killed.

There was some variation in the number of hours it took to sterilize the various cultures. In some cases, the organisms were killed in less than twenty hours, whereas in others, it took slightly over thirty hours. In Chart 2, the results of the tests in which the inoculum contained more than 1,000,000 bacteria are shown. Although the final results were comparable to those in which the inoculum was small, the bactericidal effect of sulfanilamide in 20 mg. per cent at 40.5 C. varied directly to the amount of inoculum. The results of these tests demonstrate that sulfanilamide in concentration of 20 mg. per cent is bactericidal against Lancefield group B streptococci only when the incubation temperature is elevated above 40 C. In this respect, Lancefield group B streptococci are affected in the same way by sulfanilamide as the streptococci of Lancefield group A. These results further explain the failure of sulfanilamide to destroy *Str. agalactiae* infection of the udder when the drug is administered orally. Since streptococci mastitis is a nonfebrile infection, and since the limit of tolerance for the cow for sulfanilamide administered orally is a level of 20 mg. per cent in the blood and milk, it is not possible to destroy the streptococci in the udder.

Agar Cup-Plate Method.—To simulate conditions comparable to those of the cow's udder when sulfanilamide is introduced directly into the lactiferous sinus, the following tests were conducted with the agar cup-plate method. Each culture of *Str. agalactiae* used in these tests was run in duplicate. In one Petri dish, the number of organisms in the inoculum was ten times that of the companion Petri dish. One milliliter of the diluted culture was placed in each Petri dish and 30 cc. of tryptose agar were poured on and thoroughly mixed with the culture. A sterile marble was placed in the center of the agar. When the agar hardened, the marble was removed, and the cup formed by the marble was filled with approximately 1 cc. of 37.8 per cent homogenized suspension of sulfanilamide in liquid petrolatum. The preparation of the homogenized sulfanilamide has been described in a previous publication.[3] The Petri dishes were then placed in the in-

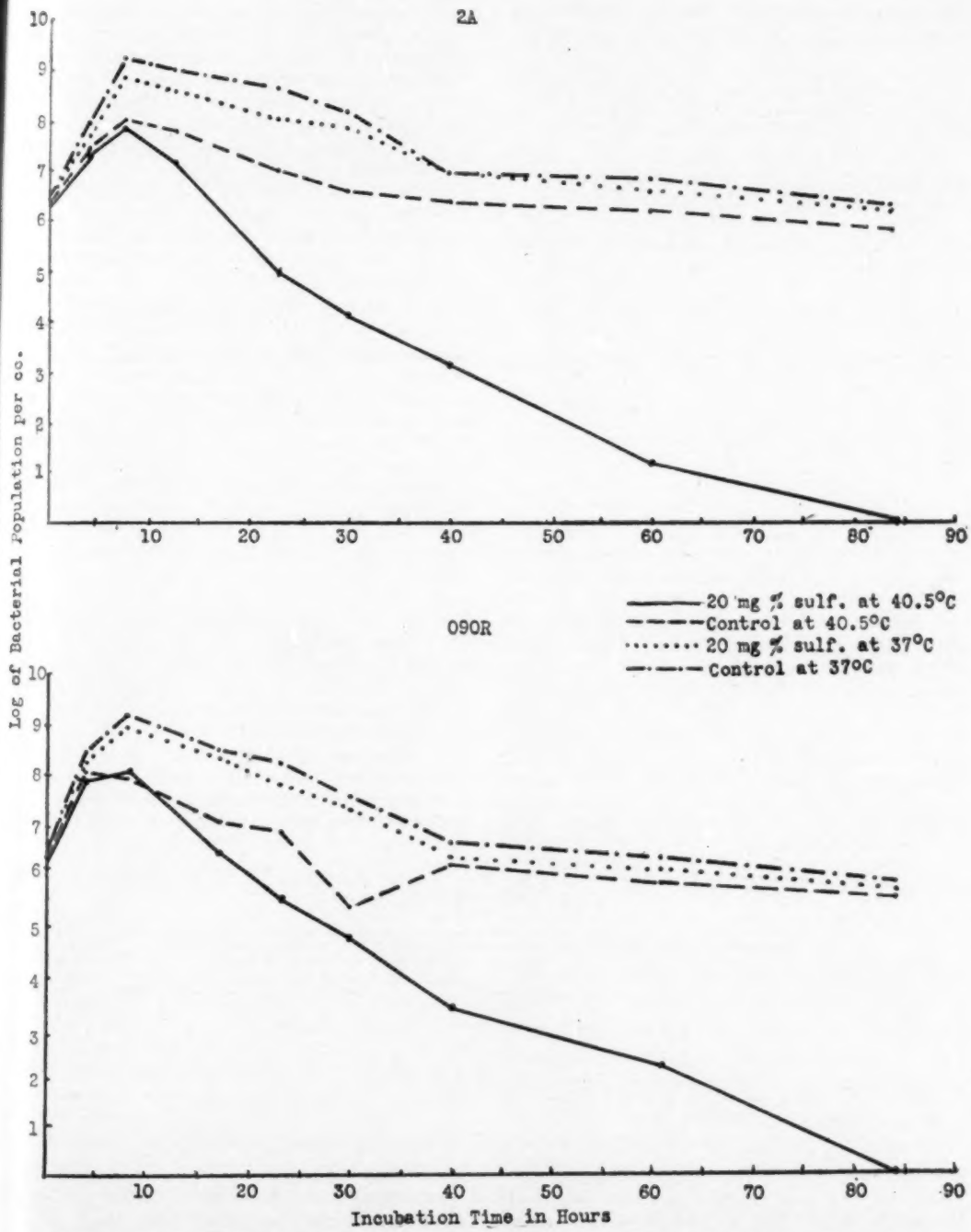

Chart 2—Effect of Sulfanilamide on Streptococcus agalatiae in in Vitro Tests Using Large Inoculum.

cubator at 37.5 C. and at intervals of twenty-four hours, subcultures were made from the plates by cutting small pieces of agar at approximately 10 mm. and 30 mm. distance from the edge of the cup and inoculating into tryptose broth. The subcultures were then incubated at 37.5 C. and the presence or absence of growth was recorded at the end of seventy-two hours. The Petri dishes were incubated for 120 hours, and at the end of this period, the zone of inhibition was measured from the edge of the cup. The amount of sulfanilamide which diffused through the agar was determined at the end of the 120-hour period at two points; namely, approximately 10 mm. and 30 mm. from the edge of the cup. The sulfanilamide determinations were made by cutting small pieces of agar and placing them in test tubes which were immersed in boiling water to melt the agar. This was then diluted with distilled hot water sufficiently high so that when the test was completed, a reading could be made with the standard sulfanilamide tubes. The test for sulfanilamide was made according to the method of Marshall and Litchfield.[4] The sulfanilamide concentration in the agar at the end of the test period was, on the average, about 730 mg. per cent at 10 mm. distance and 400 mg. per cent at 30 mm. distance.

TABLE 2—Effect of Inoculum on the Size of Inhibition Zone by Sulfanilamide on Streptococcus agalactiae (F-12a-4))—Cup-Plate Test

Hrs. Incubation before sub-culturing	No. bacteria per Petri dish							
	348		3480		34800		348000	
	a	b	a	b	a	b	a	b
11.....	—	+	—	+	+	+	+	+
26.....	—	+	—	+	—	+	+	+
46.....	—	+	—	+	—	+	+	+
70.....	—	+	—	+	—	+	+	+
94.....	—	+	—	+	—	+	+	+
125.....	—	+	—	+	—	+	+	+
142.....	—	+	—	+	—	+	+	+
Inhibition Zone.	25 mm.		16 mm.		12 mm.		8 mm.	

*a = Results of subcultures made from Cup-Plate at 10 mm. from edge of cup.
**b = Results of subcultures made from Cup-Plate at 30 mm. from edge of cup.
+ = Viable organisms present.
— = No viable organisms.

The results of this study are summarized in table 1. It will be noted that sulfanilamide was bactericidal against all the strains studied, as shown by the inhibition zone around each cup. It should be noted, too, however, that the size of the zones varies with the different strains. The smallest inhibition zone was that of strain 1B with a 6 mm. radius, and the largest inhibition zone was that of strain 090R with a 25 mm. radius. It should be further noted that the Petri dishes containing the smaller inoculum developed larger zones of inhibition compared to those with larger inoculum. This is particularly well demonstrated in table 2.

In an attempt to find an explanation for this variation in inhibition as a result of the difference in the size of the inhibition zones, the following cup-plate experiment was conducted. A series of Petri dishes were prepared in the same manner described previously, using strain F-12a-4 as the test organism. The plates were incubated at 37.5 C. and the sulfanilamide-oil preparation was added at different intervals in the agar cup. In table 3, the result of this test shows that when the organisms had an opportunity to grow for a period of approximately eight hours before sulfanilamide was added in the cup, no inhibition zone developed around the cup. It would appear, therefore, that when the organisms are allowed to multiply without the interference of sulfanilamide during the early growth stages, bacterial metabolites accumulate in the medium which nullify the bactericidal effect of sulfanilamide. The difference in susceptibility of the various strains to sulfanilamide may be explained on the basis of the above findings; namely, that group B streptococci produce a sulfanilamide inhibiting substance or substances and that some strains produce more active or more abundant inhibiting metabolites than others.

TABLE 3—The Inhibitory Effect of Sulfanilamide by Streptococcus agalactiae (F-12a-4) Metabolites Determined by the Cup-Plate Test

Petri Dish No.	Hours Incubated Before Adding Sulfanilamide	Subculture Growth Before Adding Sulfanilamide	Subculture Growth After Adding Sulfanilamide	Size of Inhibition Zone
1	0	+	—	15 mm.
2	3	+	—	8 mm.
3	8	+	+	0
4	19	+	+	0
5	33	+	+	0
6	Control	+	+	0

Number Bacteria per plate = 1620.

P-Aminobenzoic Acid and Sulfanilamide Activity for Group B Streptococci.—The problem of the nature of the bactericidal action of sulfanilamide has been made much clearer by Woods and Fildes[5] who discovered that p-ami-

nobenzoic acid* possesses antisulfanilamide activity. It subsequently was shown by Woods[6] that yeast extracts contain a sulfanilamide inhibiting substance, and he presented evidence to indicate that the yeast factor may be p-aminobenzoic acid. Fildes[7] considered that p-aminobenzoic acid was an essential metabolite for bacteria. Rubbo and Gillespie[8] have claimed the recovery of p-aminobenzoic acid from *Clostridium acetobutylicum* and showed that the acid acts as a growth factor for this organism. Since it may be possible that Lancefield group B streptococci may also produce this acid as an essential metabolite, the following experiment was conducted to determine whether p-aminobenzoic acid exerts the same inhibitory effect on sulfanilamide for group B streptococci as it does for other bacterial species.

TABLE 4—Antisulfanilamide Activity of P-Aminobenzoic Acid for Streptococcus agalactiae in Tryptose Broth

	Tube Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Sulfanilamide-mg. %	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0	0
P-aminobenzoic Acid-Gm...	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	0.01	0	0.01	0
F-12a-4 Culture—1:100,000.	0.1cc.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Growth in Original Tubes..	—	—	—	—	+	+	+	+	+	+	—	+	+

TABLE 4A—Results of Subcultures Made from Above Tubes into Tryptose Broth

Hours Incubated Before Subculturing	Tube Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
11	—	—	—	—	—	—	+	—	+	—	—	+	+
24	—	—	—	+	+	+	+	+	+	+	—	+	+
48	—	—	+	+	+	+	+	+	+	+	—	+	+
72	—	—	—	—	+	+	+	+	+	+	—	+	+
96	—	—	—	—	+	+	+	+	+	+	—	+	+
123	—	—	—	—	+	+	+	+	+	+	—	+	+

A series of 13 tubes were set up, each containing 10 cc. of tryptose broth. Into each of the 11 tubes, 100 mg. of sulfanilamide was added. P-aminobenzoic acid in increasing amounts was also added, the first tube receiving 0.001 Gm., and the tenth, 0.01 Gm. Tubes 11, 12, and 13 served as controls for sulfanilamide, p-aminobenzoic acid and tryptose broth, respectively. Strain F-12a-4 was used as the test organism. Approximately 240 bacteria were added in each tube. The tubes were then incubated at 37.5 C., and at 24-hour intervals subcultures were made into tryptose broth, using a 4 mm. loop.

Results

The data presented in tables 4 and 4A show that 0.005 Gm. of p-aminobenzoic acid was sufficient to nullify the bactericidal effect of 1,000 mg. per cent sulfanilamide. In the sulfanilamide control tube (No. 11), which contained 1,000 mg. per cent of the drug, the organisms were killed within a period of eleven hours or less. The maximum concentration of p-aminobenzoic acid (0.01 Gm.) used in this experiment had no inhibitory effect on the organisms. In table 4A, in which the results of the subcultures are recorded, it will be noted that 0.003 Gm. and 0.004 Gm. of p-aminobenzoic acid exerted partial inhibition on sulfanilamide activity since the organisms were not killed until after forty-eight hours incubation. When p-acetylaminobenzoic acid was substituted for the p-aminobenzoic acid, the sulfanilamide activity was not inhibited. These results are shown in table 5. It is of interest to note that the loss of activity of the acetylated form of the acid corresponds to the loss of the bactericidal activity of the conjugated sulfanilamide. These findings lend further support to the theory that sulfanilamide exerts its bacteriostatic action by competing (on account of similarity in molecular structure) with p-aminobenzoic acid in some enzymatic reaction essential to growth.[7] Further studies in the field of the antisulfanilamide factors and the possible discovery of substances which will neutralize their activity, should lead to valuable and far reaching results.

Discussion

Although it is not possible at present to formulate a concise explanation for the mode of action of sulfonamide drugs on microörganisms, certain facts have been re-

*The p-aminobenzoic acid used in this work was supplied by the E. I. duPont de Nemours and Company.

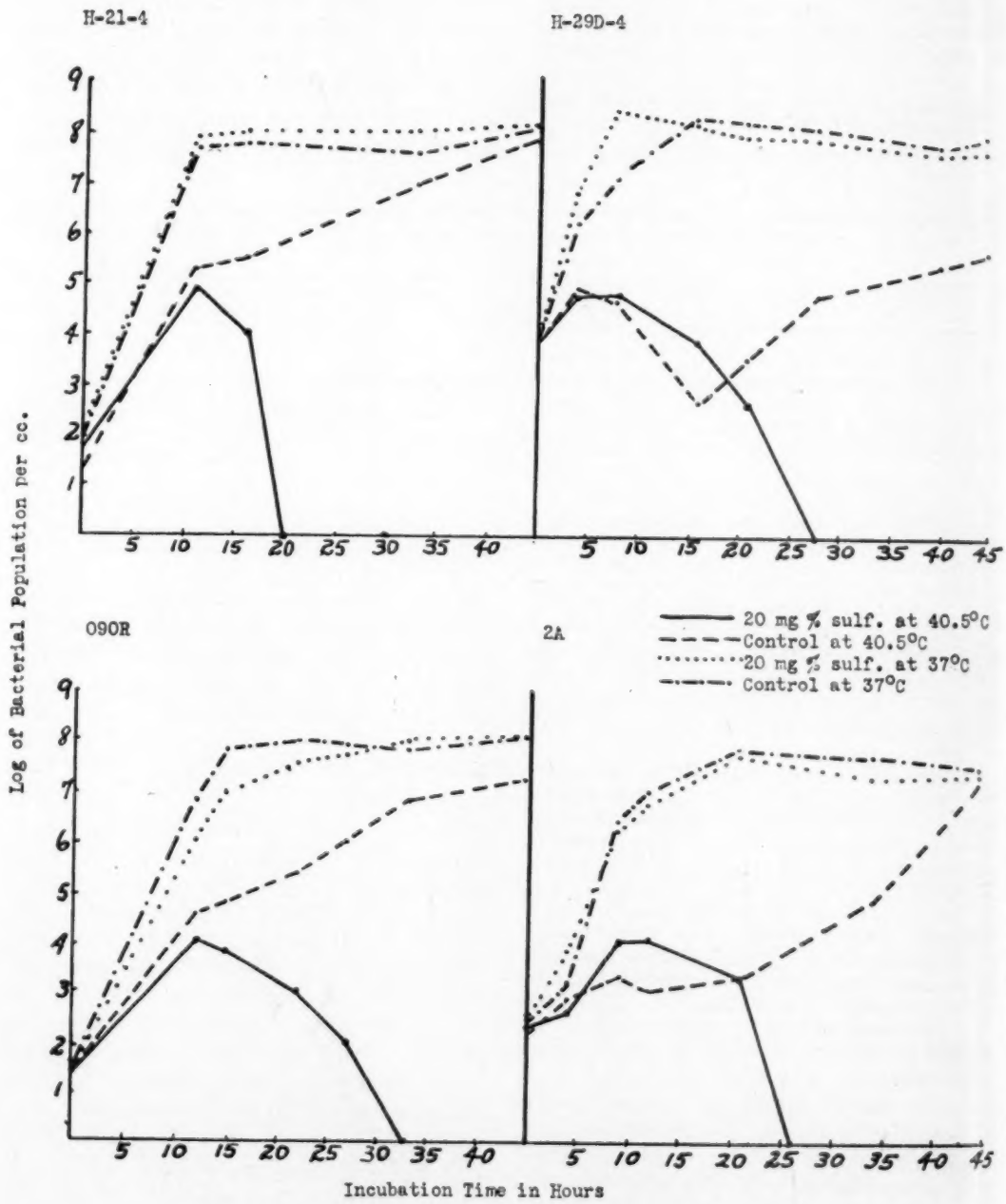

Chart I—Effect of Sulfanilamide on Streptococcus agalactiae in Vitro Tests

vealed which have an important bearing on the use of these drugs in chemotherapy. In the light of the results presented in this study, at least three factors must be taken into consideration in the treatment of *Str. agalactiae* infection of the bovine udder. In the first place, at normal body temperature the teat duct, and the clinical results of those studies have been reported in a previous publication.

In addition to the temperature and the drug concentration, a third factor has been discovered which influences the activity of sulfanilamide. Among the many bacterial

TABLE 5—Effect of P-Acetylaminobenzoic Acid on Sulfanilamide Activity for Streptococcus agalactiae in Tryptose Broth

	Tube Number										
	1	2	3	4	5	6	7	8	9	10	11
Sulfanilamide-mg. %	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0	0
P-acetylaminobenzoic Acid-Gm.	0.0001	0.001	0.005	0.008	0.01	0.015	0.02	0.025	0	0.025	0
F-12a-4 Culture-1:100,000	0.1cc.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Growth in Original Tubes	—	—	—	—	—	—	—	—	—	+	+

Results of Subcultures Made from Above Tubes into Tryptose Broth

Hours Incubated Before Subculturing	Tube Number										
	1	2	3	4	5	6	7	8	9	10	11
11	—	—	+	—	+	—	—	+	—	+	+
24	—	—	—	—	—	—	+	+	—	+	+
48	—	—	—	—	—	—	—	+	—	+	+
72	—	—	—	—	—	—	—	—	—	+	+
96	—	—	—	—	—	—	—	—	—	+	+
123	—	—	—	—	—	—	—	—	—	+	+

(37 C.) sulfanilamide in the concentration of 20 mg. per cent is not germicidal against *Str. agalactiae*. A rise of three to four degrees above the normal temperature is required to destroy these organisms with 20 mg. per cent sulfanilamide. Since streptococci mastitis is usually a nonfebrile infection, and since it is not possible to raise the sulfanilamide level in the body of the cow much above 20 mg. per cent without producing serious damage to the animal, the oral administration of sulfanilamide is of little or no therapeutic value in the treatment of streptococci mastitis.

In the second place, evidence is presented to show that sulfanilamide is germicidal for *Str. agalactiae* at 37 C. in concentrations above 100 mg. per cent.* By analogy it may be assumed that since a high level of sulfanilamide concentration is undoubtedly established in the mucosa of the stomach and small intestine when the drug is given orally, similar levels may be attained in the mammary tissue if the drug is given *via* metabolites, p-aminobenzoic acid, which is claimed to be an essential growth-substance for bacteria and which Ansbacher[9] claims to be a vitamin of the B complex group, has been found to be a sulfanilamide inhibiting substance. From the experimental data presented in this report, *Str. agalactiae* produces metabolites which counteract the bactericidal effect of sulfanilamide. No attempt was made to identify the nature of this inhibiting substance. However, p-aminobenzoic acid was found to nullify sulfanilamide activity against organisms of the Lancefield group B in the same manner as that manifested for the other bacterial species that have been reported.

In vitro experiments conducted in this laboratory have shown that considerable variation exists in susceptibility to sulfanilamide by these organisms; some strains were killed with 100 mg. per cent; others required 800 mg. per cent.

Summary

1) The results of these *in vitro* tests reveal that, at body temperature (37 C.), sulfanilamide in a concentration of 20 mg. per cent has no bactericidal effect against Lancefield group B streptococci. However, a concentration of 20 mg. per cent of sulfanilamide will destroy these streptococci at a temperature of 40.5 C.

2) In order for sulfanilamide to be bactericidal against group B streptococci at

37.5 C., it is necessary to have a concentration of the drug of over 100 mg. per cent and this high drug level must be attained during the early bacterial growth phase.

3) P-aminobenzoic acid has been found to counteract the germicidal properties of sulfanilamide on the Lancefield group B streptococci. Under the conditions of these experiments, it was found that one part by weight of p-aminobenzoic acid nullified the germicidal effect of 25 parts by weight of sulfanilamide for strain F-12a-4.

4) It appears from the results of these experiments that the therapeutic basis of sulfanilamide for Lancefield group B mastitis streptococci is based on at least three factors: the temperature of the animal at the time of treatment; the concentration of the drug at the focus of infection; and the sulfanilamide inhibiting substances which may be present in the infected area.

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Latex in the Preparation of Corrosions of Mammary Glands

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DURING THE course of extensive investigations on the utilization of sorghum feeds by dairy cattle,[1] the authors became interested in preserving excised mammary glands in a manner which would facilitate anatomical studies of the tubulo-alveolar structures in these glands.

Illustrations in the literature indicated that beeswax, paraffin, plaster of Paris,[2] and celluloid[3] were among the injection materials which were more or less unsatisfactory for the preparation of corrosion specimens of udders. Acetone solutions of vinylite resin have proved useful for studying vascular structures.[4] However, the authors found the use of vinylite in udders fraught with difficulty because the solutions failed to solidify properly after injection *via* the teat canals. Subsequent experiments have shown that it is a relatively easy matter to prepare satisfactory corrosions of the milk-containing structures of the udder by using neoprene latex.

MATERIALS AND METHODS

Corrosions have been made of udders removed from a number of different animals thus far. These include several dairy cows, some beef cows, two ewes, a heifer fetus removed from a dairy cow after eight months of pregnancy, and a Percheron mare. Small mammary glands were selected for much of this work in order to use minimum quantities of latex in developing experimental procedures.

In preparing the corrosions, the freshly excised udder is suspended in its natural position from a wooden frame. This is followed by milking the udder as thoroughly as possible with the aid of teat cannulas and udder massage. A weak acid solution, made by adding approximately 10 cc. of glacial acetic acid to a liter of water, then is injected into each gland until the gland begins to fill perceptibly with fluid. This acid solution is allowed to drain out immediately through teat cannulas. After at least two hours or over night, any remaining acid solution is removed as completely as possible by milking the udder after each of several kneadings.

Latex is injected into the udder, following irrigation with the weak acid solution. This is accomplished by securely connecting one teat cannula at a time with a glass tube extending through a three-hole rubber stopper to the bottom of a pressure jar filled with latex. The three-hole stopper is tightly secured in the mouth of the pressure jar. Two short pieces of glass tubing are fitted into the stopper, in addition to the long tube extending to the bottom of the pressure jar. To one of the short pieces of tubing is attached a hose leading to a source of compressed air. With the cannula tied firmly in place in the teat and with a moderate amount of compressed air directed into the jar, the attendant's index finger is pressed over the outer end of the second piece of short tubing in the stopper, thus permitting the compressed air to force latex through the cannula and into the gland. The rate of injection is regulated by manual control of the air pressure in the pressure jar. Injection proceeds rather rapidly at first after which the rate is reduced as the gland fills. Injection is continued until the lobules become hard, and sufficiently distended to be visibly outlined through the skin on the udder. More complete filling of the gland with latex seems to result when the injection is continued over a period of fifteen to twenty minutes rather than for shorter intervals. When the gland seems to be filled, the teat is tied off tightly to avoid leakage of latex. No attempt has been made to measure the injection pressures, but experience has shown that care is needed to avoid ruptured tubules due to excessive pressures.

The individual glands can be filled in succession until the entire udder is injected. The udder then is immersed in commercial hydrochloric acid for about two weeks or until corrosion is complete. The corrosion time can be shortened somewhat by occasional agitation of the udder in the acid. When acid maceration is finished, the udder is washed in running water for an hour or whatever time is needed to remove the acid and pieces of adhering tissue. The latex corrosion then can be immersed in a weak solution of formalin in water for display purposes, or it can be dried and

Contribution No. 157, from the Department of Dairy Husbandry, and Contribution No. 98, from the Department of Veterinary Medicine.

All latex preparations supplied through the courtesy of the Rubber Laboratory, E. I. DuPont de Nemours & Company, Wilmington, Del.

FIG. 1—Latex corrosion of teat and other milk-containing structures in a ewe's mammary gland. Tubule detail is absent in the illustration because these latex structures are so fine and flexible that they matt together except when immersed in water.

displayed as such if some darkening of the resulting cast is not objectionable.

The material used in these studies was neoprene latex. The manufacturers supplied it uncompounded, as well as compounded with dyestuffs and other substances, for this experimental use.

RESULTS AND DISCUSSION

Experiments in progress since 1941 show that neoprene latex can be used to prepare corrosions of the milk-containing structures of mammary glands. The resulting

casts, some of which are illustrated in figures 1, 2, 3, and 4, are noteworthy because of the minuteness of detail, apparent upon macroscopic examination. Further inspection of the lobules under low magnification revealed structures suggesting that the latex penetrated even into the spaces of microscopic size. The extensive detail seen in these corrosions indicates that neoprene latex is superior to other substances thus far employed to illustrate tubulo-alveolar development in mammary glands.

Uncompounded neoprene latex, designated as type 57 by the manufacturer, surpassed all the other experimental latex preparations used thus far in penetrating the finer milk-containing structures of the udder. The corrosions prepared with this type of latex were somewhat lacking in rigidity, so that it was found helpful to immerse them in water in order that the structures might assume more nearly natural positions; moreover, keeping the casts immersed in liquid avoids the darkening effect which occurs when the dry casts are exposed to air. In all other respects, latex 57 seemed to be ideal for udder injection purposes, although it should be pointed out that no attempt was made to verify the statement[5] that latex molds shrink about one in six parts so that molds should be expanded to allow for this shrinkage whenever duplication of size is an objective. The results were particularly striking when a yellow pigment was included in the latex injected into the tubulo-alveolar system, with contrasting colors of red latex in the arteries and blue latex in the veins of the udder. A modified latex, capable of producing a more rigid cast, was used for some of the injections, but it failed to penetrate the finer udder structures as well as type 57. Type 571 produced a rigid cast less subject to fragmentation during handling, but it also failed to penetrate some of the finer spaces in the udder.

Preliminary work with these latex casts shows that they are useful tools in studying udder structure. Observations have been made, for example, on the gross arrangement of the tubules by progressively shearing away parts of the latex cast. These studies are being continued. Already it appears from work of this general character that relatively little is known about the actual structure of the bovine udder either in normal or in diseased individuals,

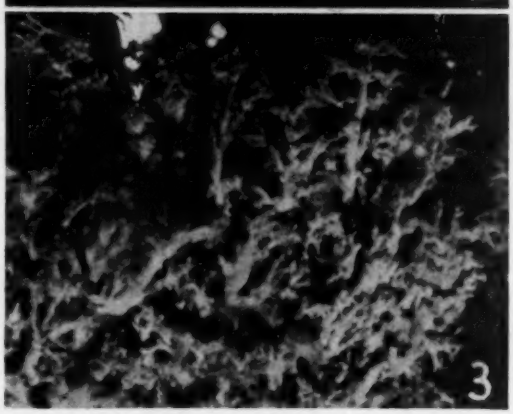

FIG. 2—Enlargement of a fragment of latex corrosion removed from one of the lobules of the gland shown in fig. 1. Note the divided and redivided tubules together with the abundant grapelike structures.

FIG. 3—Portion of a photograph of a latex corrosion made from one quarter of a dairy cow's udder. Note the tree-like appearance of the tubular development.

and that certain statements in the anatomical literature may need revision and extension.

The use of latex for injection purposes evidently started about five years ago.[6]

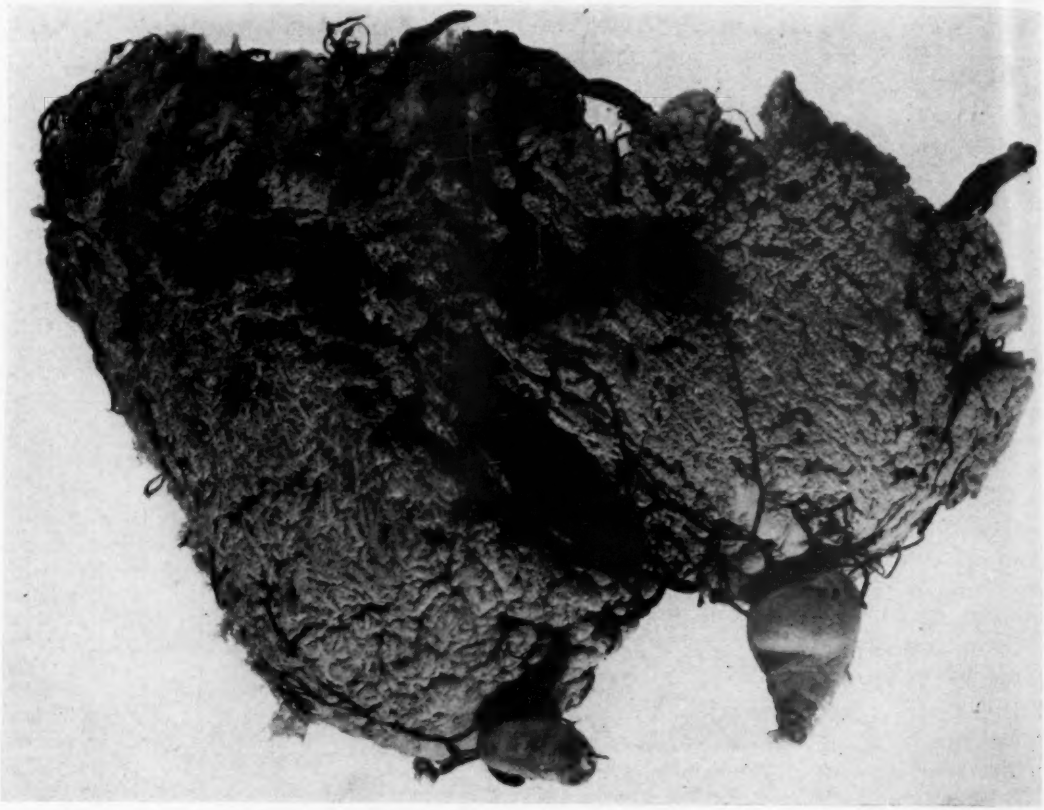

FIG. 4—Latex corrosion of the right half of an udder removed from a dairy heifer which died from a foreign body about one month before her first calving. Note general arrangement of the milk-containing structures. The vascular system was injected with red latex (arteries) and blue latex (veins).

Since that time, it has been used frequently in researches on the circulatory system, including that of the bovine udder.[7] However, in so far as the authors are aware, this is the first time that anyone has employed latex for the preparation of corrosions of the milk-containing structures of mammary glands. The results thus far have been gratifying not only to the authors but also to dairy specialists, veterinarians, and others who not infrequently have indicated their previous lack of appreciation of the intricate system of structures exhibited by these latex corrosions.

SUMMARY AND CONCLUSIONS

A procedure is outlined for injecting mammary glands with neoprene latex (Du Pont) after the freshly excised glands have been irrigated with a weak acid solution.

Corrosions remaining after maceration of the injected glands in hydrochloric acid reveal anatomical arrangements of the milk-containing structures in greater detail than that shown by any other known injection material used in connection with comparable techniques.

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Studies on Bovine Gastrointestinal Parasites VIII; Effects of a Low Plane of Nutrition on Immunity to the Stomach Worm Haemonchus Contortus

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SINCE the demonstration (Mayhew[1] 1941) that a definite immunity can be established to further infection with the large stomach-worm, *Haemonchus contortus*, by preceding infection, further study of several phases of the subject has been under way. In the following pages is given a description of certain experiments in which the effects of a low plane of nutrition were studied in its effect on the persistence of the already established immunity.

The procedure followed in the experiments was to use calves, in which a definite resistance had been shown to exist, and feed them cotton seed hulls only, except for a small amount (1/2 to 1 pt.) of commercial calf concentrate scattered on top of the hulls to encourage the animals to eat at the regular feeding times. In general, the calves ate the hulls very readily but got tired of the monotonous diet and became restless at the time the other calves were being fed unless given this small amount of concentrate. Usually, within approximately a month, the animal would begin to show definite effects of the poor feed in loss in weight and general muscular weakness, but no indication of vitamin deficiencies were observed in any of the animals. After definite effects were produced, test inoculations of larvae were given and the animals watched closely and the hulls supplemented with a small amount of hay and concentrate if their strength declined too rapidly, so that death would not result before the end of the experiment.

The methods used in handling and feeding, before the feeding of hulls, were the same as used in the experiments reported in the preceding papers of this series of which a description is given in Mayhew.[2,3] The method of fecal examination is the same as used in all the other experiments of this series, a description of which, with an analysis of data, is being prepared in a separate report.

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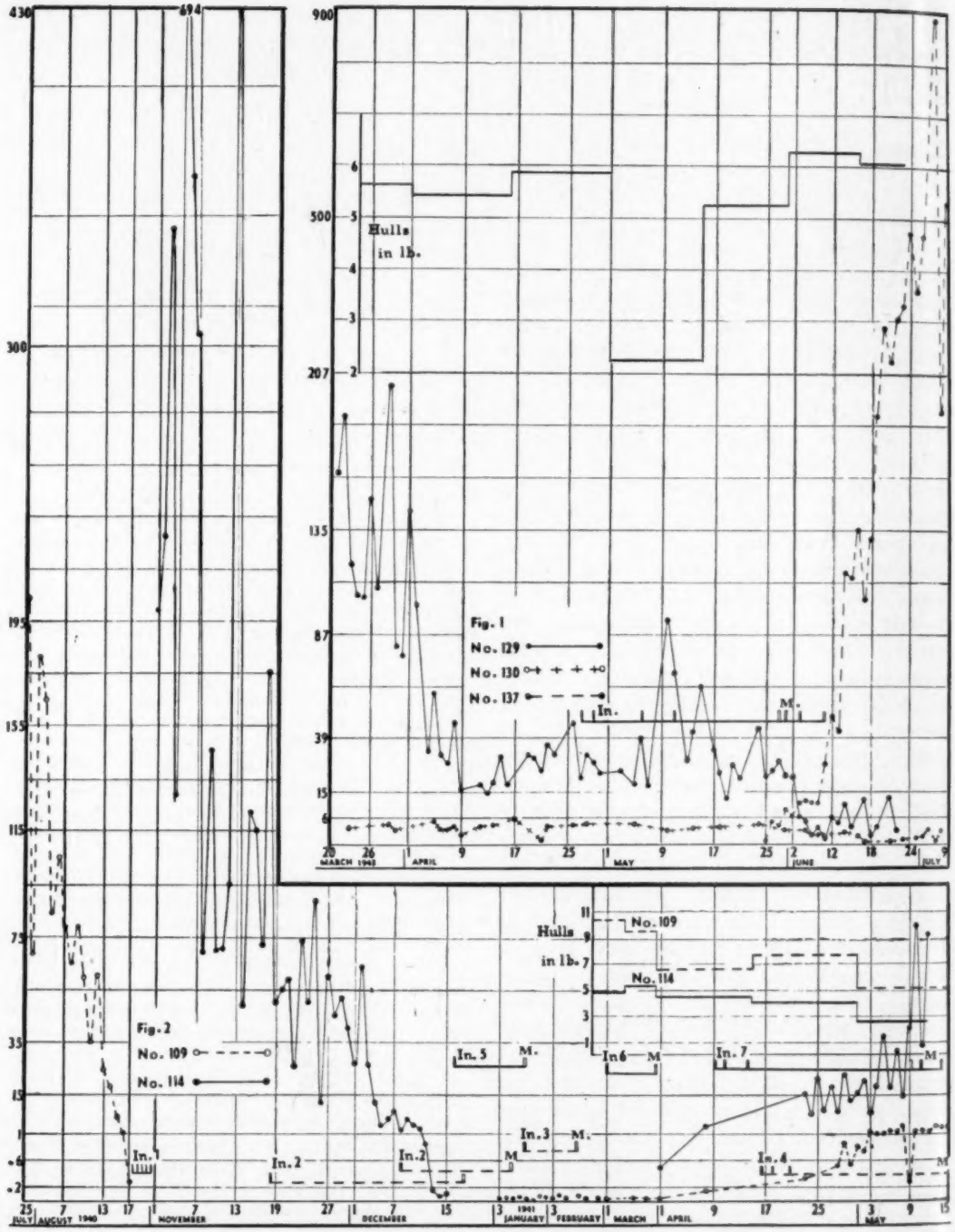
REVIEW OF LITERATURE

An attempt will not be made in this short paper to give a complete discussion of all the references to dietary deficiencies and parasitism, but only to mention some which might be especially interesting. The papers of Ackert and others working in his laboratory describe the relationship of various dietary supplements to infection with the chicken nematode, *Ascardia lineata*, and in this connection Ackert and Beach[4] will be helpful. Foster and Cort[5,6] describe experiments that indicated that a generally deficient diet rendered dogs more susceptible to infection with the dog hookworm, *Ancylostoma caninum*. Chandler[7] working with *Nippostrongylus muris* in rats reports, "diet was found to play no part in the acquisition or retention of worms as long as the animals thrived on it,—but rats kept on a diet of coarse vegetables and fruit on which they grew thin to the point of emaciation—were found to have a tendency to acquire more worms from a given inoculation, and to harbor them for a longer time, than did rats on more favorable diets." Porter[8] says, "it is possible for more adults of *Nippos. muris* to develop in rats on a diet of whole milk than in those on the regular stock ration. This phenomenon occurred both with a primary infection where only the host's natural resistance was present and with reinfection where an acquired resistance was present." Riley[9] and Watt, Golden, Olason and Mladinich[10] report a decrease in resistance to *Nippos. muris* infection in rats on a diet deficient in vitamin A.

In using the bovine host as an experimental animal, we are, of course, unable to answer many questions about details of what is actually lacking in the diet used and what specific substances the lack of which caused the extreme emaciation and even death. We note, also, with special interest in this connection



In. = Inoculation, date indicated by short vertical line.

M. = Maturing date of larvae—end of prepatent period 28 days after inoculation.

H. = Date on which feeding of bulls was begun.

E.P.G. = Eggs counted per gram of sediment remaining after final centrifugation; no screening or sampling being done in the procedure.

the work of Wegner, Booth, Elvehjem and Hart[11] in which it has been shown that the vitamin B complex is synthesized in the rumen, which may explain why indication of vitamin deficiency was not observed.

EXPERIMENTAL DATA

Calf 129.—This animal was a grade Jersey male born Nov. 11, 1941. He was given the first infective Haemonchus larvae on Aug. 28, and Sept. 1, 1942, and the resulting egg count ranged between 200 and 1500 eggs per gram of sediment (not shown in fig. 1). Two additional series of inoculations were given as follows: the first, Nov. 24, 28, Dec. 8, 10, 1943; and the second, Jan. 12, 16, 21, and 30, in order to establish an immunity. The egg count dropped as a result from the previous high range to a maximum of about 200 eggs per gram of sediment and the feeding of cotton seed hulls was begun on March 24, 1943 (fig. 1—hulls in lb.). There was a further decrease in the egg count range to between 15 and 90 as indicated in figure 1. It will be noted (fig. 1) that the average number of pounds of hulls consumed was above 5.5 during April, and that there was a sudden drop (fig. 1) to 2.25 lb. At the same time, there was a very rapid decline in the strength of the calf and it was necessary to supplement the hulls with some hay and concentrate in order to prevent the death of the animal. The animal showed marked improvement by May 15, and the inoculations designed to test the immunity were given April 27, 29, May 6 and 11, 1943 (fig. 1). It will be noted that, instead of an expected increase, the egg count decreased to a lower level; no count above 14 was obtained after June 2 (fig. 1).

An equal amount of the same suspension of infective larvae was given to calf 137, a nonimmune calf born Jan. 28, 1943, at the same time as that to calf 129 in order to check on the vitality of the larvae given to calf 129. Since calf 137 was a much younger animal (born Jan. 28, 1943), he was not eating as much hay as calf 129; consequently, the egg counts are considerably higher than would be expected in a calf the same age as calf 129 (fig. 1). It does show, however, that the larvae administered were good infective larvae.

The egg counts of calf 130 shown in figure 1 are included because this gives an opportunity to show data on this animal for two months preceding that shown in figure 4. He was kept under the same conditions as were calf 129 and 137 at this time.

That the cotton seed hulls were a very poor source of food elements is indicated by the weights of calf 129. On March 23 he weighed 405 lb.; on April 6, 360; April 30, 345; and on June 21, 360 lb. The animal was found dead on June 22, and upon postmortem examination the tissues were normal in appearance and a relatively small number of *H. contortus* (129 males and 448 females) were recovered.

Calf 109.—This animal was a grade Jersey male born Dec. 16, 1939. The results of the first series of inoculations in which immunity was established have been reported (Mayhew[1]—fig. 5). To demonstrate the existence of an immunity, 5 additional inoculations (In. 1—fig. 2) were given on Aug. 23, 24, 27, 28, and 30, 1940, and two more on November 18 and December 9 (In. 2—fig. 2). After August 17 the egg count became 0, with the result that between August 17 and December 29, 91 fecal examinations were all negative during the 167-day period (fig. 2). At the end of the prepatent periods of the larvae administered on November 18 and December 9, a few eggs began to appear in the manure samples after January 1, but the egg count remained under 0.08 egg per gram of sediment during the next three months, even though an additional inoculation was given on January 17 (In. 3—fig. 2).

The feeding of hulls was begun on February 22 (fig. 2), and the last inoculations to test the immunity were given on April 16, 18, and 22, 1941 (In. 4—fig. 2). The egg counts increased to between 1 and 10 eggs per gram of sediment, indicating that only a few larvae were permitted to develop. Daily egg counts were made between April 27 and June 14, not all of which are shown in figure 2. A portion of this increase is due to the decrease in the amount

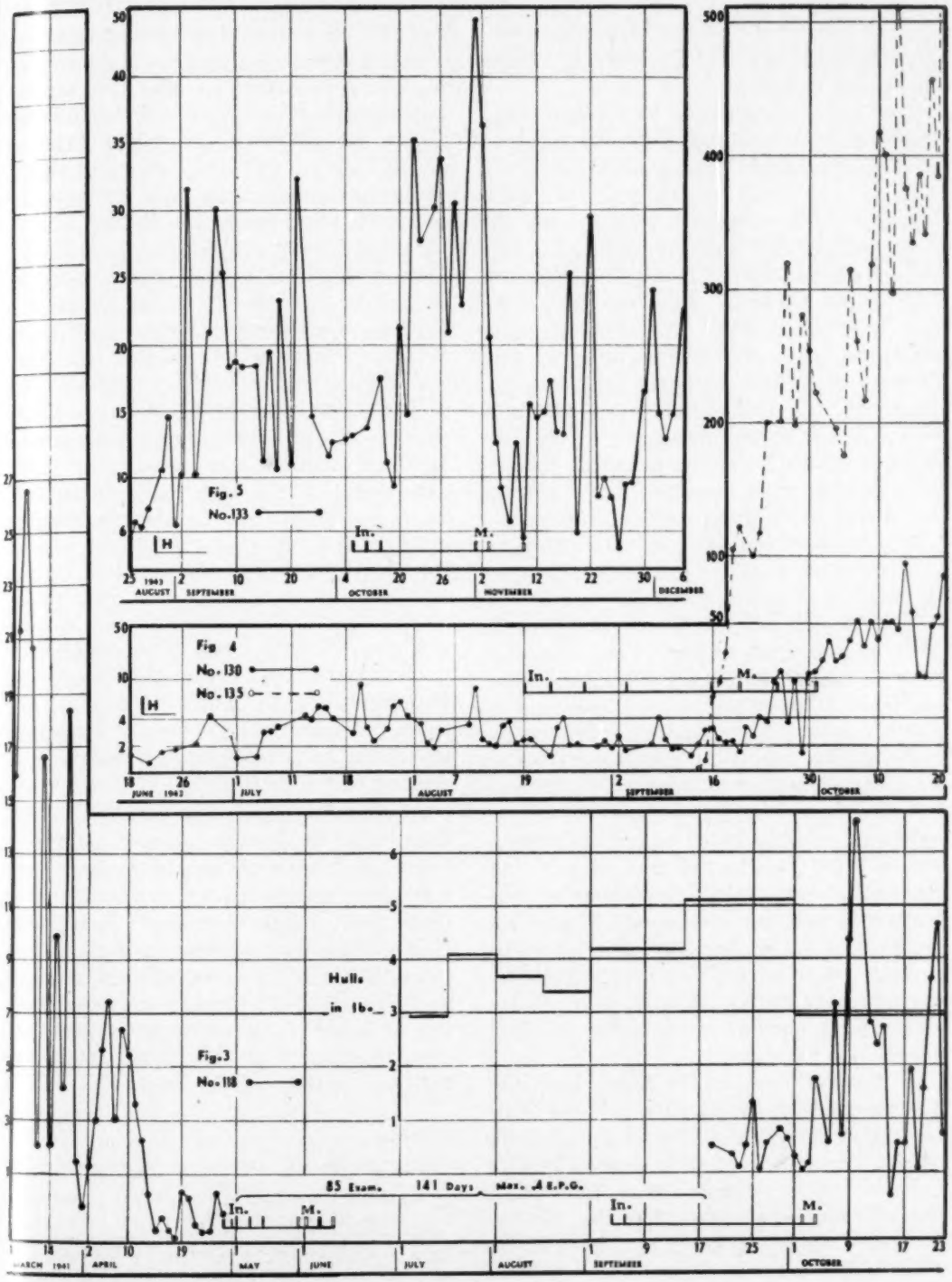
of hulls consumed, 7.7 to 5.0 lb. per day (fig. 2). That the hulls were a very poor feed is indicated by the decrease in weight from 515 lb. on February 21 to 457 lb. on April 29, a loss of 58 lb., and the animal was in a very much weakened condition by April 29. This animal was not sacrificed because further observations were desired in his particular case.

Calf 114.—This animal was a grade Jersey male born April 16, 1940. He was given a single heavy inoculation of Haemonchus larvae on Sept. 4, 1940, with the result that a relatively high egg count developed; the details of which have been reported previously (Mayhew[1]—fig. 6). On Dec. 16, 1940, an additional inoculation (In. 5—fig. 2) was given to determine if immunity had been established with the result that, in the period of 105 days from the middle of December until April 1, the maximum egg count did not exceed 0.35 eggs per gram of sediment in the 54 examinations made. On March 1 an additional inoculation (In. 6) was given the calf with the result that the egg counts increased to an average of about 15 eggs. On February 22 the feeding of cotton seed hulls was begun, and three additional inoculations were given on April 9, 11, 14, 1941 (In. 7) to test the effects of the poor feed on the established immunity. Toward the end of the prepatent period of twenty-eight days (M.), there was an increase in the egg counts to from 15 to 80 eggs per gram of sediment. A portion of this increase was due to the decrease in the amount of hulls consumed, a decrease of from 4 to 2.5 lb. per day. During the time of the experiment, the weight of the calf decreased from 240 lb. on February 21 to 171 lb. on May 12. On May 12, 1941, the calf was so weak that it was necessary to end the experiment. On postmortem examination, a relatively small number of *H. contortus* (92 males and 101 females) was recovered and the tissues of the digestive organs were normal in appearance except for slight edema of the mesentry about the fourth stomach.

Calf 118.—This animal was a pure bred Jersey male born Aug. 22, 1940. He received the first Haemonchus larvae on Jan. 27, and 28, 1941, as a result of which 33 egg counts, made between February 25 and March 31, varied between 2 and 26 eggs per gram of sediment. Following this, there was a decline in the counts (fig. 3); 14, made between April 1 and 14, varied between 0.5 and 7.5, and between April 15 and 30 none of the 16 counts made were above 0.75 eggs per gram of sediment. Four very heavy inoculations were given on April 25, 29, May 5, and 10, 1941 (fig. 3) in order to test the degree of resistance that seemed to have been established. During the following 141 days from May 1 to September 18, 85 examinations were made and the maximum egg count obtained was 0.4 egg per gram of sediment.

The feeding of cotton seed hulls was started on July 1 (fig. 3), and two inoculations were given on September 4 and 6 (In.—fig. 3) to test the persistence of the immunity. It will be observed that there was an increase in the egg counts to between 1.0 and 3.5 eggs during the last half of September due to early maturing of larvae (prepatent period is fourteen to twenty-nine days, Mayhew[1]), and a further increase during October to a range of 0.7 to 14 eggs per gram of sediment, indicating that there has been some breakdown in the resistance to the invading larvae. A large portion of the last increase is evidently due to the decrease in the amount of hulls consumed since it will be noted (fig. 3) that the amount consumed decreased from an average of 5.17 lb. per day during the last half of September to 2.9 lb. during October, with the result that his strength declined very rapidly during this time until his death on the night of Oct. 23, 1941.

At the beginning of the hull feeding experiment, the calf weighed 314 lb. on July 3. On September 5, his weight was 262 lb., and at the time of his death on October 23, he weighed 178 lb. On postmorten examination there was no external evidence of parasitism and, grossly, the tissues appeared normal except for the extreme degree of emaciation. Sixteen male and twenty-one female *H. contortus* were recovered from the fourth stomach. Other species of parasites were absent. At no



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E.P.G. = Eggs counted per gram of sediment remaining after final centrifugation; no screening or sampling being done in the procedure.

time in the course of the experiment was any diarrhea or other evidence of severe parasitism detected.

Calf 130.—This animal was a pure bred Jersey male born Nov. 8, 1941. He received the first infective stomach worm larvae on Aug. 28 and Sept. 1, 1942, and the resulting egg count range was rather high, between 81 and 777 eggs per gram of sediment between October 1 and November 20. During the following six weeks, there was first a rather sudden drop followed by a gradual decrease to less than 10 eggs. The feeding of hulls was begun on June 23, 1943 (fig. 4). His decline in strength was so rapid that it was necessary to add 3 to 4 lb. of hay and about a pound of concentrate for the next seventeen days in order to prevent too rapid a decline in strength.

The larvae for the test inoculations were cultured from a calf with a mixed infection of stomach worms and nodular worms and were given on Aug. 19, 23, 28, and Sept. 3, 1943, and an equal amount of the same suspension was given to calf 135 as a control on the viability of the larvae. Increases in egg counts due to the maturing of stomach worm larvae, therefore, should have begun by about September 16, and from the nodular worm larvae about September 26 in both animals. It will be noted (fig. 4) that there was a rapid rise in the case of calf 135, the nonimmune control, beginning on September 16 and not in the case of calf 130 until about September 26. This later rise in calf 130 to as high as nearly 100 eggs can probably safely be attributed to the maturing of nodular worm larvae to which he was not resistant.

At the beginning of the experiment, calf 130 weighed 520 lb. on June 21. On October 20, at the close of the experiment, his weight was 490 lb., thus, instead of making regular gains during the four-month period, there was a loss of 30 lb. He was in a very much weakened condition at the close of the experiment, and was not killed because it was desired to use this animal in further experiments.

Calf 133.—This calf was a grade Jersey male born June 9, 1942. He received the first infective stomach worm larvae on Nov. 14, 1942, and the resulting egg count ranged between 3 and 44 eggs per gram of sediment, except for one count of 103. Two additional series of inoculations were given, the first on March 2, 4, 17, and the second July 2, 6, 9, 1943, without changing the egg counts range and without any noticeable effect on the calf indicating a resistance to stomach worm larvae. The feeding of the hulls was begun (H—fig. 5) on August 29. The final test inoculations were made on October 5, 7, and 13 (In.—fig. 5). There was no essential change in the egg counts range even though the period of observation extended until December 15 and about thirty days beyond the time when the last larvae given should have matured. (M.—fig. 5). This calf was not killed because we wished to use him in additional experiments.

SUMMARY

A description is given of six experiments in which attempts were made to break down an established immunity to the stomach worm, *Haemonchus contortus*, by placing the calves on a low plane of nutrition by feeding cotton seed hulls.

In three of the experimental animals, there was no increase in the degree of infection judging the results by egg counts following reinoculation; while in three there was a slight increase. It has been shown (Mayhew[1]), however, that a slight increase occurs in some animals when on good feed; but it is never possible to establish as heavy an infection by reinoculation after immunity as has been established with the initial inoculation.

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Experimental Intracerebral Infection of Horses, Cattle, and Sheep with the Virus of Vesicular Stomatitis

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VESICULAR STOMATITIS in horses and cattle is characterized by an acute rise in temperature followed by the formation of vesicles and erosions on the epithelium of the tongue and mucous membrane of the oral cavity. Occasionally, lesions also occur on the teats and rarely on the feet. No disturbance of the central nervous system has been observed following natural exposure.

There are two recognized, immunologically distinct types of virus causing this disease, the Indiana and the New Jersey[1,2].

Cox and Olitsky[3,4] first demonstrated the potential neurotropism of this virus by the intracerebral inoculation of guinea pigs and mice, and, by selection, developed a neurotropic strain of each of the two types of the virus.

This paper covers the results of the intracerebral injection of these neurotropic strains of vesicular stomatitis virus in horses and cattle, the chief hosts of the disease, and in sheep, a species naturally resistant.

MATERIALS AND METHOD

Dried mouse brain suspensions (10 per cent in hormone broth), containing vesicular stomatitis virus with established neurotropic properties, were kindly supplied by Dr. P. K. Olitsky of the Rockefeller Institute for Medical Research and were used by us in initiating these studies. These viruses (New Jersey and Indiana strains) were originally isolated from natural cases at the Bureau of Animal Industry, Experiment Station, Bethesda, Md. The New Jersey and Indiana viruses had been frozen and dried forty-one months and thirty-nine months, respectively. Both strains were resuspended in distilled water and passed simultaneously through mouse brains and guinea pig pads.

From the Animal Disease Station, Beltsville, Md., (Frank and Appleby) and Pathological Division, U. S. Bureau of Animal Industry, Agricultural Research Administration, Washington, D. C., (Seibold).

Material from the fifth guinea pig and seventh mouse passages of the New Jersey virus and the first and fifth guinea pig and sixth and eighth mouse passages of the Indiana virus was used to initiate the tongue and intracerebral inoculations of horses, cattle, and sheep, and the intranasal inoculation of a colt. Guinea pig pad suspensions were prepared by grinding infected pads in a mortar and suspending the ground material in physiologic salt solution (0.5 to 1 cc. per pad). Mouse brain emulsions were prepared by grinding infected brains in a mortar with sterile sand and suspending the material in salt solution in proportion of 1 cc. per brain for regular mouse passages, and in various proportions by weight for the large animals. This suspension was centrifuged at a slow rate of speed for ten minutes and the supernatant fluid used for the intracerebral inoculations.

The horses used for this work had been exposed two to four months previously to infectious anemia and had passed through two to four definite temperature reactions prior to the vesicular stomatitis inoculations. The cows and sheep were normal. The temperature of each animal was taken twice daily.

With the exception of four cases, the mouse and guinea pig brain suspensions of virus used for intracerebral inoculations of large animals were titrated by the intracerebral inoculation of mice. Serial dilutions for titrations were made in the arithmetical complement multiple of 10, and 2 mice were injected with each dilution. All mice received a constant amount of fluid or 0.03 cc. of the supernatant fluid or serial dilution. The maximum dilution causing death of both mice or death of 1 mouse and severe reaction in the second mouse was designated one minimal lethal dose (m.l.d.).

Material from the tongue lesions and from the brains of large animals was tested for the presence of virus by inoculating the metatarsal pads of normal guinea pigs and, in most instances, the brains of normal mice. The identification of virus type was established by cross immunity tests on guinea pig pads.

The absence of bacterial contamination in brain suspensions was determined by the usual cultural methods.

Routine histologic examination was made of the brains of 4 horses, 2 cows, and 2 sheep. The tissues examined were taken usually from

several areas in the cerebrum, cerebellum, and brain axis; fixed in formalin; embedded in paraffin, and stained with hematoxylin and eosin. Special neurohistologic techniques were not employed in this study.

Cow 2334.—On Dec. 31, 1942, cow 2334 received, intracerebrally, 1 cc. of a mouse brain suspension of New Jersey virus prepared by grinding two infected brains with 2 cc. of physiologic saline. The mouse brain suspension was not titrated. On the following two

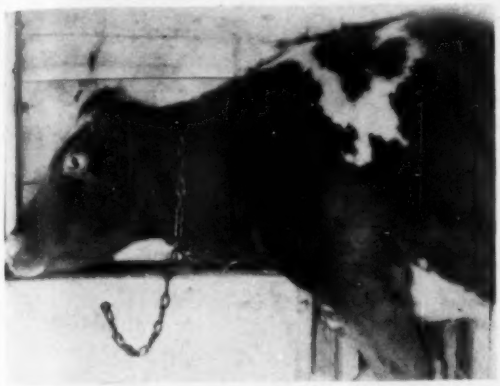

Fig. 1—Cow 2334 in a clonic spasm that recurred at irregular intervals on the third day after intracerebral inoculation.

days she appeared nervous and had a constant tremor of the skin of the forelegs and over the withers. On Jan. 3, 1943, when excited, the cow would instantly develop a clonic spasm and, at irregular intervals, spasms occurred without any apparent external stimulus. During the periods of excitement, she assumed a pose of bracing her legs and pushing her head against the front of the stall (fig. 1) for about twenty minutes at a time. This was followed by relaxation, first accompanied by tremors, and then leading to more complete relaxation. Her temperature rose to 103.6 F. The next morning she was down with a temperature of 104.4 F. Her legs were held extended and in constant motion. She was killed in the afternoon of the fourth day and her brain, about 18 in. of cervicothoracic spinal cord, 50 cc. of citrated blood, and 50 cc. of cerebrospinal fluid were removed. These organs and fluids were sterile when cultured for bacterial contamination.

The washed red blood cells, cerebrospinal fluid, spinal cord suspension, cerebellar suspension, and cerebral suspension were made up in various dilutions and titrated intracerebrally in mice. The mice which received the blood, cerebrospinal fluid, and spinal cord suspensions, respectively, showed no reaction. Those which received suspensions of cerebellum died from the 10^{-1} through the 10^{-3} dilutions, and those which received suspensions of cerebrum died from the 2 by 10^{-1} through the 2 by 10^{-5} dilutions. Guinea pig pad inoculations with cerebrospinal fluid and the cerebellum were positive, but were negative for the other tissues.

Histologic examination of the brain (fig. 2) revealed a nonpurulent encephalitis characterized by disseminated lesions of inflammatory and degenerative character. There was a moderate degree of lymphocytic, perivascular infiltration of scattered blood vessels throughout the brain. However, a number of sections showed little definite alteration. There was also a mild degree of inflammatory cellular infiltration of the meninges. The most pronounced alterations were degenerative changes involving the neurons in scattered areas of the

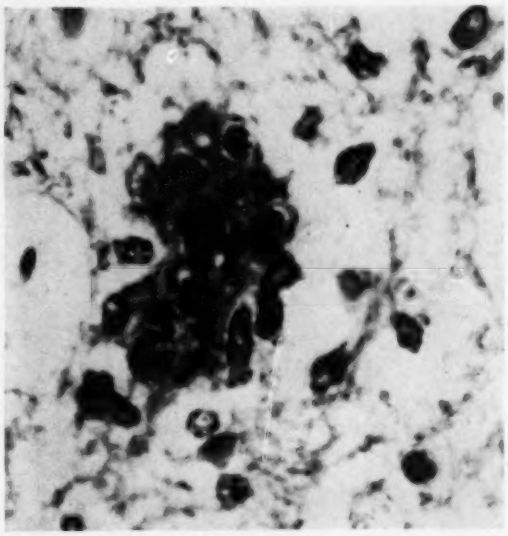

Fig. 2—Cow 2334. Neuronophagia. Brain stem. x 800.

cerebral cortex and the brain axis. These changes were rather marked in some regions, having terminated in necrosis of the cells. A moderate degree of polymorphonuclear leucocytic infiltration and also accumulations of microglia cells were present in the tissue more often in the areas containing degenerated or necrotic neurons. There was considerable neuronophagia, by both microglia cells and polymorphonuclear leucocytes. Areas of degeneration in the white matter as observed in cow 2327 (described later) were present in this case only to a limited extent. In general, the lesions in this case were less pronounced and extensive than in cow 2327.

Cow 2322.—On Dec. 31, 1942, cow 2322 was inoculated on the tongue with a suspension of guinea pig pads infected with the New Jersey type of the virus. No rise in temperature was observed. On Jan. 2, 1943, she developed two

large vesicles at the site of inoculation. The coverings of these vesicles were removed and stored in the refrigerator in 50 per cent glycerin-phosphate solution buffered to pH 7.6. On Feb. 22, 1943, she was inoculated intracerebrally with 1 cc. of a 1:10 suspension of mouse brain derived three passages previously from the cerebellum of cow 2334 which had succumbed to intracerebral New Jersey virus inoculation. The virus content of the 1:10 suspension of mouse brain was 30,000,000 m.l.d. per cc. On the following day her temperature rose to 103.6 F. and on Feb. 24, 1943, to 105.8 F. Following this, it gradually receded until it reached a normal level of 101.6 F. on March 1, 1943. A loss of appetite during the height of the febrile reaction was the only other abnormality in the clinical picture. To test the susceptibility of this animal to the Indiana type of the virus, she was inoculated intracerebrally on April 27, 1943, with 2 cc. of a 1:5 suspension of infected guinea pig brain which contained 600 m.l.d. per cc. This inoculation appeared to have no effect. Eleven months later she developed lesions on the tongue from a local inoculation with the Indiana virus.

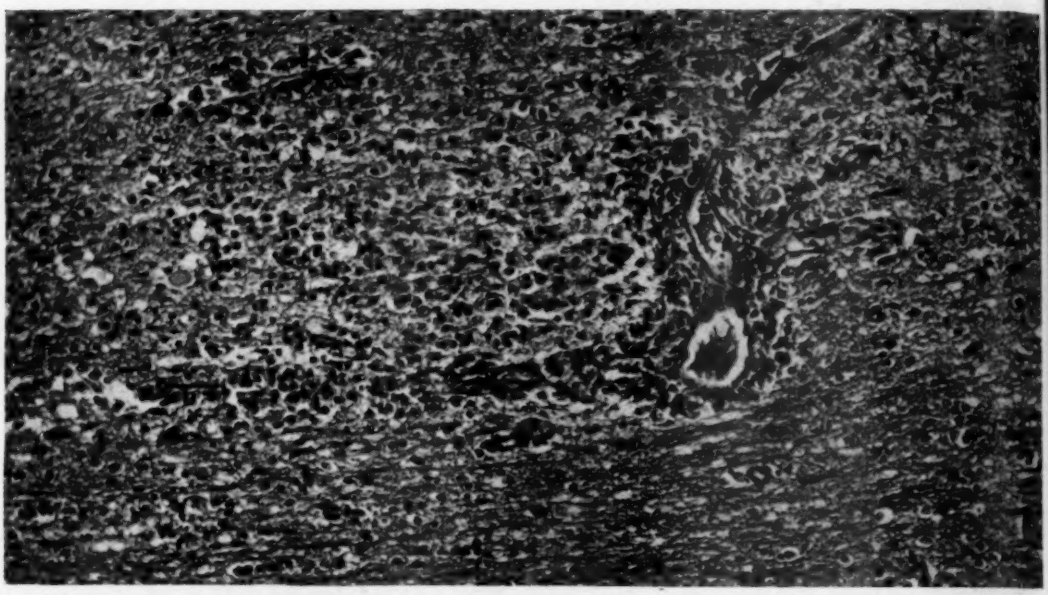

Fig. 3—Horse 1016. Focus of acute inflammation showing perivascular infiltration and accumulation of inflammatory cells in the adjacent tissue. Cerebral white matter. The picture includes an entire focal lesion. x 150.

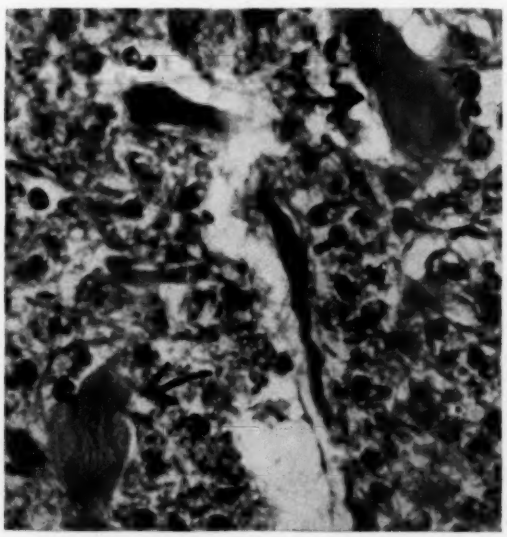

Fig. 4—Horse 1016. Arrows point to swollen degenerated axones. There is also some inflammatory cellular infiltration. Brain stem. x 475.

Mare 1016.—On Jan. 12, 1943, mare 1016 was injected intracerebrally with 1 cc. of a 1:4 dilution of New Jersey virus infected mouse brain suspension. The 1:4 dilution contained 750,000 m.l.d. per cc. Within twenty-four hours her temperature had risen to 102.2 F. and remained above normal except for two days preceding death, at which time it had receded to normal. On Jan. 15, 1943, she was more excitable than usual, and on Jan. 16, 1943, she developed a slight stagger. On the following day there was a more pronounced stagger and she ceased eating and drinking. On Jan. 18, 1943, she was down, but with much difficulty arose and remained on her feet the

next day. At this time, when forced to move her gait simulated stringhalt. On Jan. 21, 1943, she was down with paralysis of the extremities. She died the next day, ten days following inoculation. Postmortem study revealed extensive changes in the viscera, mainly of a hemorrhagic type, which resembled that of acute infectious anemia. This pathologic condition was probably due chiefly to the fact that this animal suffered from infectious anemia. It has been observed that occult infections with infectious anemia virus are sometimes activated by artificial exposure to equine encephalomyelitis virus[5]. The latent, infectious anemia virus in this case may likewise have been activated by the stomatitis virus. That the virus of vesicular stomatitis was present in this case was shown by the fact that the guinea pigs inoculated metatarsally with suspensions of cerebellum and cerebrum developed typical vesicular lesions. Mice succumbed to intracranial injections with heavy brain suspensions.

Histologic examination of the brain (fig. 3 and 4) revealed a disseminated, nonpurulent encephalitis characterized by large foci of inflammatory and degenerative changes scattered through the cerebrum and the brain axis. The lesions were usually in close relation to certain vessels or groups of vessels, which showed some degree of perivascular infiltration. Degeneration of the tissue in the affected areas was evident and necrotic neurons were readily observed in some of the foci involving the grey matter. Numerous microglia

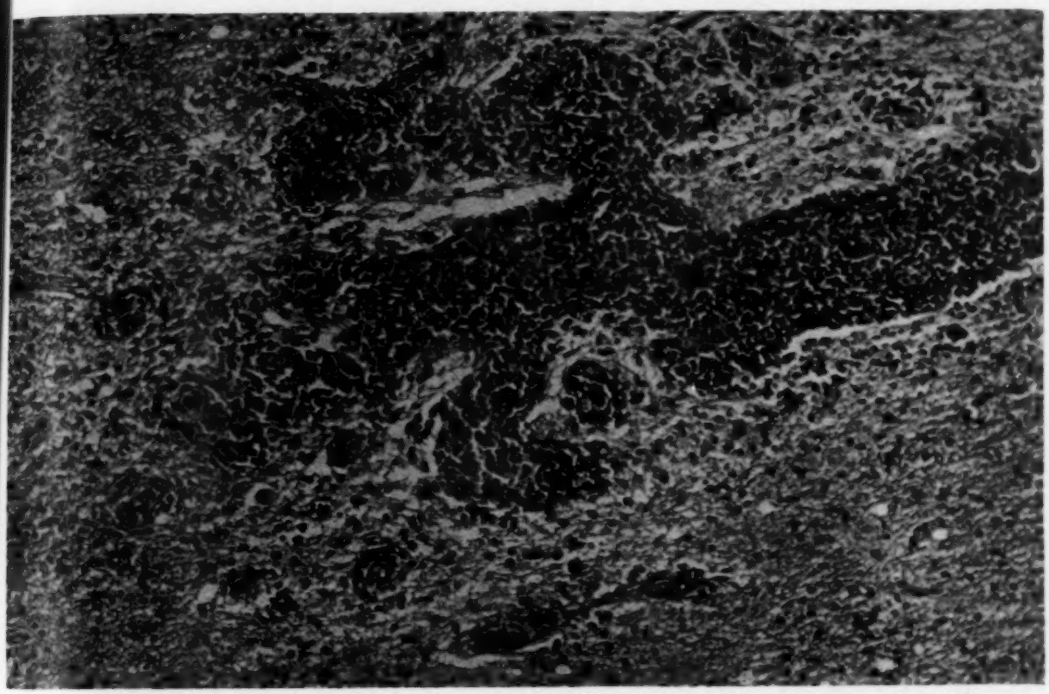

Fig. 5—Colt 1354. Perivascular infiltration and rarefaction of the adjoining tissue—cerebral white matter. The picture includes almost an entire focal lesion. x 150.

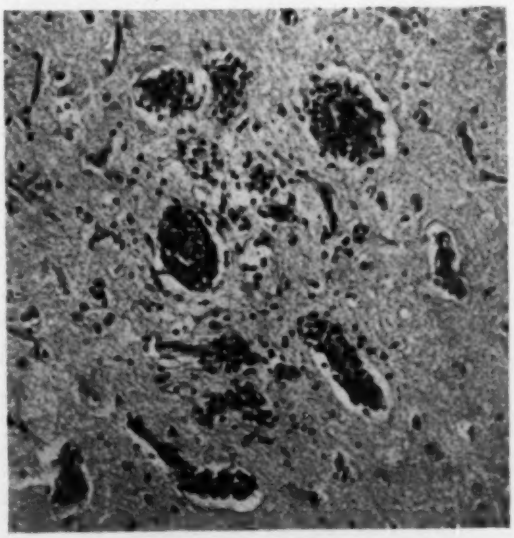

Fig. 6—Colt 1354. Perivascular infiltration with slight rarefaction and cellular infiltration of the adjoining tissue. Cerebral grey matter. x 150.

cells were present in the lesions. Varying amounts of cellular infiltrate, consisting mainly of monocytes and macrophages, were present. In some instances, the latter cells were quite numerous, indicating a large amount of tissue destruction. The brain, outside the inflammatory areas, showed little deviation from the normal state.

Colt 1354.—When mare 1316 was inoculated on the tongue with the New Jersey strain of the virus on Jan. 7, 1943, her suckling; colt 1354, was kept in the same stall with her. He developed no apparent lesions of vesicular stomatitis, but had a slight febrile reaction on the fourth and fifth days following inoculation of the mare. On Feb. 22, 1943, he was inoculated intranasally. One cc. of a 1:10 suspension of mouse brain (30,000,000 m.l.d. per cc.) derived three passages previously from the cerebellum of cow 2334 which had succumbed to an intracerebral New Jersey virus inoculation, was sprayed onto the nasal mucosa. No apparent lesions or symptoms such as temperature reactions developed. From April 9, 1943 to April 26, 1943, the colt passed through four febrile reactions each lasting one to three days. On April 27, 1943, he was inoculated intracerebrally with 1.5 cc. of a 1:5 dilution of guinea pig brain suspension which contained 600 m.l.d. of Indiana virus per cc. There was a slight temperature reaction twenty-four to forty-eight hours following injection, but on the sixth day there was a continuous reaction that existed for eight days. That this may have been caused or partially caused by infectious anemia is suggested by the fact that the colt had a second continuous febrile reaction that lasted for six days, which began on the twentieth day following the eight day reaction. The temperature was slightly higher during the first eight day period than during the second six-day period. Also the febrile reactions previous to inoculation were characteristic of infectious anemia. The colt was killed seven weeks after the intracerebral injection. Brain suspensions were negative for the Indiana virus when inoculated into mice and guinea pigs.

Histologic examination of the brain (fig. 5 and 6) revealed scattered foci of chronic inflammatory and degenerative change involving the vicinity of single vessels and groups of vessels. A few lesions were present in the cerebral cortex, but a majority of the foci were found in the white matter of the cerebrum and the brain axis. The affected vessels showed perivascular accumulations of small lymphocytes, macrophages, and adventitial cells. In some lesions, the perivascular accumulations were so pronounced as to give the vessels a markedly thickened appearance. The degenerative changes consisted of a usually narrow zone of rarefaction surrounding the infiltrated vessels. In some regions in the cerebral white matter, several smaller foci of rarefaction had fused to form a larger and more diffuse type of lesion. Only a limited amount of cellular infiltration of the rarefied parenchyma was present. The brain tissue outside the focal lesions showed no significant change.

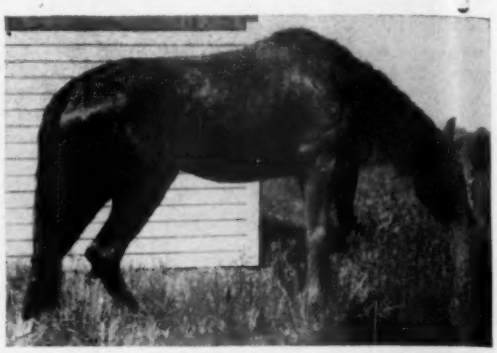

Fig. 7—Mare 1316, twelve days after intracerebral inoculation with Indiana type virus, showing muscular incoördination on forced movement.

Mare 1316.—On Jan. 7, 1943, mare 1316 was inoculated on the tongue with a suspension of the New Jersey type virus contained in the vesicle coverings removed from the tongue of cow 2322. Her temperature rose slightly on the following day. Forty-eight hours after inoculation she had developed a few small vesicles on her tongue. On Feb. 22, 1943, she was inoculated intracerebrally with 1 cc. of a 1:10 suspension of mouse brain (30,000,000 m.l.d. per cc.) derived three passages previously from the cerebellum of cow 2334 which had succumbed to an intracerebral, New Jersey virus inoculation. Following this the only apparent change in health was a slight rise in temperature that persisted for six days. On April 27, 1943, 2 cc. of a 1:5 suspension of guinea pig brain, containing 600 m.l.d. per cc. of Indiana type virus, was injected intracerebrally. The mare developed a slight temperature which persisted for the following three days. On May 6, 1943, nine days later, she had become listless and her movements were incoördinated. Forty-eight hours after the appearance of these symptoms, she appeared to be very weak and managed to stay on her feet only by leaning against the sides of the stall. On May 9, 1943, she was much stronger (fig. 7) and had begun to eat and drink for the first time in four days. Following this her recovery was uneventful. This mare was killed seven weeks after the last intracerebral injection. Brain suspensions proved negative for the Indiana virus when inoculated onto guinea pig pads.

The histologic alterations in the brain were essentially similar to those in colt 1354, with the exception that the foci of chronic inflam-

matory and degenerative change were considerably less numerous in this case. There was no significant alteration from the normal state in a majority of the brain sections. The lesions in both these cases were evidently of a residual nature.

Sheep 1889.—On Jan. 12, 1943, sheep 1889 was inoculated on the tongue with the New Jersey type virus in a suspension of material from the tongue lesions of cow 2322. No visible lesions developed (1 horse and 2 guinea pigs developed lesions when inoculated simultaneously with the same suspension). On Feb. 22, 1943, he was inoculated intracerebrally with 1 cc. of a 1:10 suspension of mouse brain (30,000,000 m.l.d. per cc.) derived three passages previously from the cerebellum of cow 2334, which had succumbed to an intracerebral inoculation with New Jersey type virus. Twenty-four hours later his temperature had risen to 106.2 F. and it remained between 104.6 and 106.4 F. until death on Feb. 28, 1943. The symptoms preceding death were those of an encephalitis, with weakness, anorexia, and incoördination with developing paralysis. When the brain was removed, putrefaction had set in and a histopathologic examination was not performed. A 33 per cent suspension by weight was made of material from the cerebellum and also from the cerebrum. These suspensions were tested for the presence of virus by the inoculation of the metatarsal pads of guinea pigs. The 4 animals used to test the cerebellar suspension developed typical vesicles at the site of inoculation; 2 animals used to test the cerebral suspension developed no lesions.

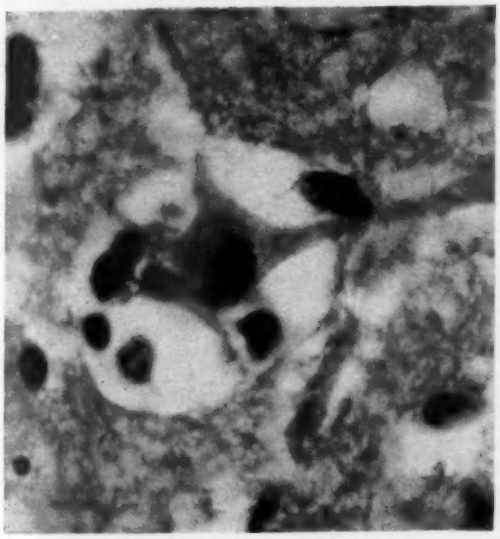

Fig. 8—Cow 2327. Necrotic neuron. Cerebrum. x 800.

Sheep 162.—On Jan. 12, 1943, sheep 162 received intracerebrally 0.75 cc. of a 1:6 suspension of New Jersey virus infected mouse brain containing 500,000 m.l.d. per cc. On Jan. 14, 1943, his temperature had risen to 105.6 F. and remained above 104.2 F. until Jan. 19, 1943, at which time the sheep went down with paralysis of the extremities. The temperature receded to 102.4 F., but again rose to 105.2 F. on Jan. 25, 1943, and remained above 103.0 F. until the animal was killed while still in a paralytic state on Jan. 28, 1943. There were no visible lesions on postmortem examination. A histopathologic examination was not made. Intracerebral inoculations of suspensions of cerebellum, cerebrum, kidney, spleen, and liver into mice gave inconclusive results; but guinea pigs inoculated meta-

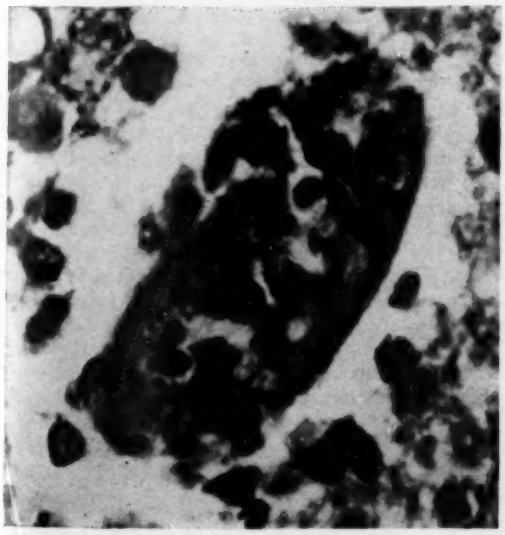

Fig. 9—Cow 2327. Neuronophagia. Brain stem. x 800.

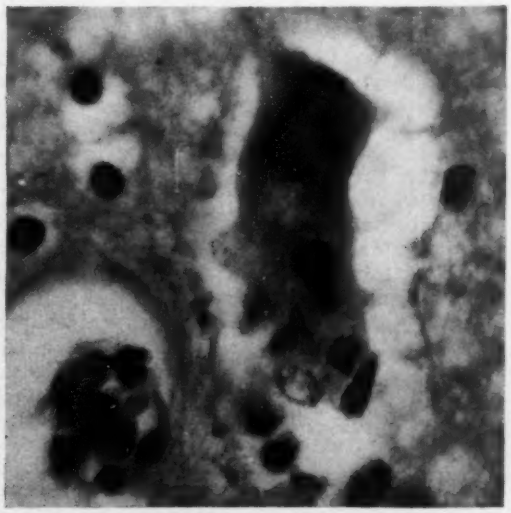

Fig. 10—Cow 2327. Necrotic neuron. Cerebrum. x 800.

tarsally with suspensions of cerebellum and cerebrum developed fair vesicular lesions.

Sheep 38.—On Jan. 12, 1943, sheep 38 received an intracerebral inoculation of 0.5 cc. of a 1:6 suspension of New Jersey virus infected mouse brain, containing 500,000 m.l.d. per cc. On the following day his temperature had risen from 101.6 to 105.6 F. and remained between 104.2 and 107.4 F. until death. General paralysis had set in on the fifth day and the sheep died on the sixth day following injection. The postmortem examination was complicated by advanced putrefactive changes, but no macroscopic lesions were found. Tests for the presence of virus and histopathologic examinations were not made.

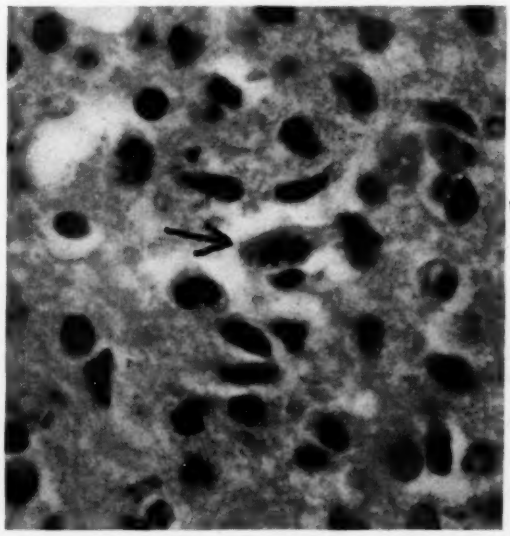

Fig. 11—Sheep 1982. Arrow points to necrotic neuron. The adjacent tissue contains numerous microglia cells. Basal ganglion. x 800.

Cow 2327.—On Jan. 12, 1943, cow 2327 was inoculated on the tongue with a suspension of metatarsal pads from the first Indiana guinea pig passage. These pads had been stored in glycerin-phosphate buffer in the refrigerator for forty days. Forty-eight hours later no lesions were evident, and it was assumed that the virus in the pads had lost its activity. Therefore, on Jan. 14, 1943, cow 2327 was reinoculated on the tongue with the Indiana type virus contained in a heavy emulsion of fresh pads from the fifth guinea pig passage. There was no rise in temperature following inoculation. On Jan. 16, 1943, one large vesicle appeared on the tongue, and material from this lesion was removed and stored in the refrigerator. On Feb. 23, 1943 the cow was inoculated intracerebrally with 1 cc. of a 1:10 suspension of infected mouse brain from the eighth mouse brain passage of the Indiana virus. This 1:10 suspension of inoculum contained 300 m.l.d. per cc. No evidence of infection occurred. The temperature remained within the normal limits of 101.0 to 101.8 F. On April 22, 1943, she was inoculated intracerebrally with the New Jersey strain of the virus. One cc. of a 1:10 suspension of a second passage guinea pig brain was used. This was not titrated. Forty-eight hours later her temperature had risen from 101.2 to 104.8 F. and remained around 105.0 F. until death. On the fourth day after inoculation she was down with paralysis of the extremities, and she died the next day, April 27, 1943, or five days after inoculation. Putrefaction had set in before the autopsy. Mice injected with Seitz filtrate from the brain did not develop encephalitis.

Histologic examination of the brain (fig. 8, 9, and 10) revealed a pronounced nonpurulent encephalitis characterized by inflammatory and degenerative changes involving both grey and white matters. There was well-marked perivascular infiltration (cuffing) of numerous blood vessels with small and large lymphocytes as well as occasional large monocytelike cells. The piarachnoid was similarly infiltrated. The most pronounced alterations were degenerative changes involving the neurons in certain areas of the cerebral cortex and the brain axis. These changes were rather advanced in character, affecting both the nucleus and cytoplasm, and in many instances it was evident that the affected neurons had become necrotic. Some neuronophagia was also observed. Focal and diffuse areas of inflammatory cellular reaction were present in the tissue in association with the degenerated neurons. This reaction consisted principally

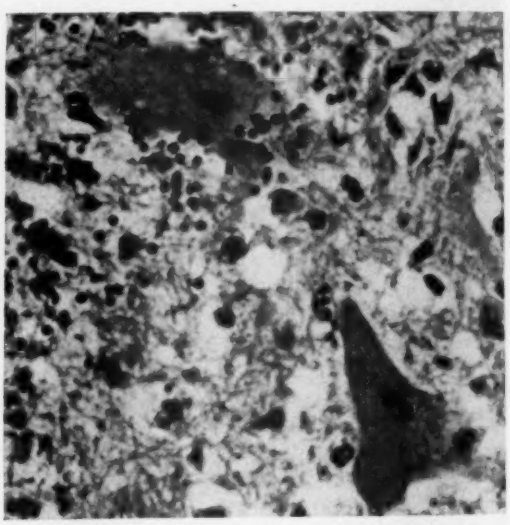

Fig. 12—Sheep 1982. Degenerated neurons and hemorrhage. Region of the fourth ventricle. x 475.

of accumulations of rod cells and monocyte-like cells suggesting mobilized microglia. Foci of degeneration involving the white matter were also observed. These appeared as areas of slight to well-marked rarefaction with associated neuroglial and microglial proliferation. Occasional deposits of granular eosinophilic material were present in the rarefied areas.

Colt 1359.—On Feb. 2, 1943, colt 1359 was inoculated intracerebrally with 1 cc. of a 1:5 suspension of pooled mouse brain from the

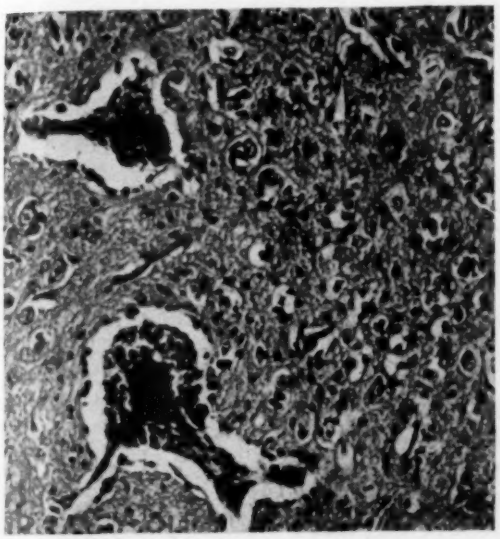

Fig. 13—Sheep 1882. Portion of a lesion in the cerebral grey matter showing perivascular infiltration and increase of cellular elements in the adjacent tissue consisting of infiltrating lymphocytes and microglia cells. x 190.

sixth and eighth passage of the Indiana virus which contained 600,000 m.l.d. per cc. This was followed by a slight febrile reaction in twenty-four hours. With the exception of four days the febrile reaction was continuous for twenty-two days. During the last fourteen days of the 22-day period, the course of the temperature reaction became undulating, normal in the morning and rising in the afternoon. Other clinical symptoms began developing about the fourth day after injection. The movements became incoördinated, anorexia began on the sixth day, paralysis of the extremities developed on the seventh day, and the colt remained recumbent for several days. After regaining its feet, the colt remained stiff legged, and walked on its toes, simulating contracture of the deep flexor tendons. Certain clinical symptoms and postmortem findings suggested that the reactions following inoculation with vesicular stomatitis may have been complicated by simultaneous infectious anemia reactions. These consisted of continued febrile reactions that occurred intermittently until the animal was killed (78 days after inoculation), and of the numerous hemorrhages of the viscera found on postmortem examination. Emulsions of the brain did not contain sufficient vesicular stomatitis virus to infect laboratory animals.

Histologic examination of the brain failed to reveal significant pathologic alterations.

Sheep 1982.—On Feb. 2, 1943, sheep 1982 was inoculated on the tongue with 1 cc. of a 1:5 suspension of pooled mouse brain from the sixth and eighth passage of the Indiana virus which contained 600,000 m.l.d. per cc. No apparent infection resulted. Reinoculation with the Indiana virus was performed on Feb. 4, 1943, with a heavy emulsion of material obtained from the tongue lesion of cow 2327. No visible lesions were produced. On April 22, 1943, this sheep was inoculated intracerebrally with 0.5 cc. of a 1:10 suspension of guinea pig brain containing New Jersey

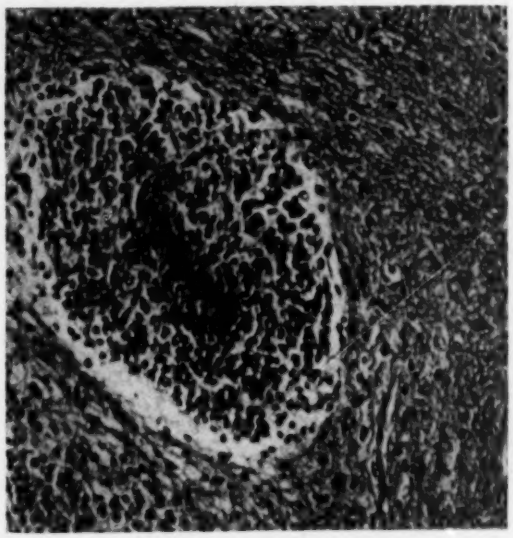

Fig. 14—Sheep 1882. Perivascular infiltration. White matter of cerebellum. x 190.

type virus. This suspension was not titrated. The temperature rose the next day and remained above normal until death. On April 26, 1943, she was down with paralysis of the extremities and her temperature had risen to 106.6 F. On the fifth day, twenty-four hours later, the temperature was 109.4 F. and the animal died during the night. By the next morning, putrefactive changes were so advanced that material used to check the presence of virus was passed through a Seitz filter. The filtrate was negative in mouse brain inoculations.

Histologic examination (fig. 11 and 12) of the brain revealed a well-marked, nonpurulent encephalitis characterized by inflammatory

and degenerative changes involving scattered areas in the cerebral cortex and the brain axis. There was a moderate degree of lymphocytic perivascular infiltration of the vessels in the affected areas. The meninges in this case, however, were heavily infiltrated with lymphocytes, monocytes, and large phagocytic cells. Degeneration and necrosis of the neurons were observed in the brain stem and in some areas in the basal ganglia and cerebral cortex. In the cerebral cortex, there were a few areas in which not only the neurons, but also the neuroglia cells and the ground substance, were undergoing retrogressive alterations. In these regions, there was little if any inflammatory, cellular reaction. Wherever present, the cellular reaction consisted of infiltrating lymphocytes and accumulations of microglia cells. Hemorrhage was noted in the meninges and in a number of the perivascular spaces.

Sheep 1882.—On Feb. 2, 1943, sheep 1882 was inoculated intracerebrally with 0.5 cc. of a 1:5 suspension of pooled mouse brain from the sixth and eighth passage of Indiana virus which contained 600,000 m.l.d. per cc. In 24 hours the temperature had risen to 106.6 F. but had gradually receded to 103.6 F. by the fifth day. No other symptoms developed. On April 22, 1943, this sheep was inoculated intracerebrally with 0.5 cc. of a 1:10 suspension of guinea pig brain from the second guinea pig brain passage of the New Jersey virus. The guinea-pig brain was not titrated. The temperature rose within twenty-four hours and remained above normal until the animal was killed. During the last week, beginning May 1, 1943, the sheep became progressively weaker, with an increasing incoördination in his gait, and inappetence on the last three days. On the sixteenth day he had become partially paralyzed and remained on his side, keeping his legs in a constant running motion. He was killed on this day. The virus was not recovered from the brain when injected intracerebrally into mice.

The histopathologic alterations (fig. 13 and 14) in this case consisted principally of occasional foci of inflammatory change involving the cerebral cortex, cerebellum, and cerebellar peduncles. Tissues from the basal ganglia were not included in the study. There was a mild degree of lymphocytic infiltration of the meninges as well as lymphocytic perivascular infiltration of occasional vessels and groups of vessels. In connection with some of the perivascular infiltration, there had been proliferation of the adventitial cells, giving the vessels a greatly thickened appearance, also indicating a less acute stage of the lesions. Lymphocytic infiltration and an increased number of microglia cells were observed in circumscribed areas, particularly in the cerebral grey matter and usually in association with the perivascular infiltration. A few damaged neurons were present in these areas, but pronounced and extensive degeneration of the nerve cells as observed in sheep 1982 had not occurred in the tissues examined in this case.

DISCUSSION

Horses, cattle, and sheep were susceptible to experimental inoculation intracerebrally with vesicular stomatitis virus. The period of incubation ranged from twenty-four to forty-eight hours. This is similar to the incubation period following local inoculation on the tongue in horses or tongue and dental pad in cattle. A rise in temperature is the first symptom in either type of inoculation.

The New Jersey type virus proved to be lethal when inoculated intracerebrally into susceptible animals (table 1). Five out of eight animals inoculated intracerebrally died. The remaining were killed *in extremis*. The course of the disease was from five to sixteen days or longer. The dominant symptoms were a rise in temperature, psychic disturbance, motor irritation, and paralysis. Most animals were unable to eat or drink a few days before general paralysis set in. All of these symptoms were present in most individuals.

Immunity to the New Jersey virus, when injected intracerebrally, was established in a horse and cow by first infecting them by local inoculation on their tongues. A sheep was resistant to local inoculation on the tongue, but succumbed later to intracerebral inoculation.

The Indiana type of vesicular stomatitis virus used in these experiments appeared to cause much milder reactions, when inoculated intracerebrally in large animals, than the New Jersey type (table 1). The original material of both types received from Olitsky was contained in infected mouse brains. Subinoculations made at this station were from mouse brain to mouse brain, and guinea pig pad to guinea pig pad, the pad material being used for local inoculations on the tongue of large animals and the mouse brains for intracerebral inoculations.

This Indiana type guinea pig pad material produced local lesions when inoculated

TABLE I.—Results of Inoculation of Cattle, Horses, and Sheep with Virus of Vesicular Stomatitis

Animal Species	Lingual			Intracerebral				Intracerebral			
	Virus	Date	React.	Virus	Date	React.	M.L.D.	Virus	Date	React.	M.L.D.
Cow 2334				N. J.	12-31-42	+ + + +	Not tested				
Cow 2322	N. J.	12-31-42	pos.	N. J.	2-22-43	+	30,000,000	Ind.	4-27-43	— (a)	1,200
Mare 1016				N. J.	1-2-43	+ + + +	750,000				
Colt 1354		Nursing No. 1316	—	N. J.	Nasal 2-22-43	—	30,000,000	Ind.	4-27-43	+	900
Mare 1316	N. J.	1-7-43	pos.	N. J.	2-22-43	+	30,000,000	Ind.	4-27-43	+ +	1,200
Sheep 1889	N. J.	1-2-43	—	N. J.	2-22-43	+ + + +	30,000,000				
Sheep 162				N. J.	1-12-43	+ + + +	375,000				
Sheep 38				N. J.	1-12-43	+ + + +	250,000				
Cow 2327	Ind.	1-12-43	—	Ind.	2-23-43	—	300	N. J.	4-22-43	+ + + +	Not tested
Colt 1359	Ind.	1-14-43	pos.	Ind.	2-2-43	+ + +	600,000				
Sheep 1932	Ind.	2-2-43	—	N. J.	4-22-43	+ + + +	Not tested				
Sheep 1882	Ind.	2-4-43	—	Ind.	2-2-43	+	300,000	N. J.	4-22-43	+ + + +	Not tested

(a) = 11 months later was found to be susceptible to Indiana virus by lingual inoculation. pos. = vesiculation. — = negative to virus infection.
+ = rise in temperature only. + + = incoördination. + + + = paralysis of extremities. + + + + = paralysis and death.

on the tongue of 1 cow only (cow 2327). When inoculated lingually in 1 horse, 1 colt, and 1 other cow, this virus failed to produce lesions. However, the epithelium at the site of inoculation on the tongue of the cow did cause well-developed lesions when inoculated on the tongues of other cattle and horses. These inoculation tests are not reported in the body of the paper.

On the other hand, similar material of the New Jersey type virus was found capable in all cases of causing local lesions on the tongues of horses and cattle. Neither virus produced tongue lesions in sheep.

An explanation of the difference in virulence of these two types of vesicular stomatitis virus may be obtained from the variation in the number of m.l.d. contained in the respective inoculums used for intracerebral inoculations. In all instances in which titrations were made of the mouse brain materials, it was done simultaneously with the inoculations of the large animals. The findings, which obviously could not be determined until after the intracerebral injections had been made, showed that the Indiana inoculums contained from 300 to 600,000 m.l.d. per cc., whereas the New Jersey inoculums contained from 500,000 to 30,000,000 m.l.d. per cc.

In inoculation experiments made at this station in previous years, it was shown that local lesions on the tongue would usually develop ten to eighteen hours sooner with the New Jersey type than with the Indiana type virus. Also, in a mixed virus experiment[6] in which equal quantities of pads of guinea pigs affected with New Jersey and Indiana types of virus, respectively, were mixed and transferred serially on the pads of guinea pigs, only the New Jersey type of virus remained after the second serial passage in one instance, and after the fourth passage in another. These observations, together with the results reported in this experiment, indicate that the New Jersey type of vesicular stomatitis virus is more invasive or virulent than the Indiana type.

In Friedemann's[7] review of the permeability of the blood-brain barrier to individual neurotropic viruses, the vesicular stomatitis virus is classed as one that is

unable to pass through the capillaries of the central nervous system. Sabin and Olitsky[8] have shown that when this virus is injected into various tissues of 15-day-old mice it gains access to the central nervous system exclusively through neural paths. Intranasal inoculation of young mice led to C.N.S. infection, but mice over one year old could not be infected except by direct inoculation into the brain. In our work, however, the 10-month-old colt (1354) apparently did not become infected from its dam when the latter contracted the disease from a local exposure on the tongue (table 1). At a later date the colt was exposed by spraying the nasal mucous membrane with a suspension of New Jersey virus containing 30,000,000 m.l.d. per cc. but did not become infected.

There were no pathognomonic histopathologic alterations produced in the central nervous system by the vesicular stomatitis viruses that could be used for diagnostic purposes.

SUMMARY

The virus of vesicular stomatitis, when injected intracerebrally, was found to be infectious for horses, cattle, and sheep, resulting in a rise in temperature, psychic disturbance, motor irritation, and paralysis.

The intracerebral injection of the New Jersey type virus proved lethal for horses, cattle, and sheep. Following recovery from a local inoculation on the tongue, a horse and cow were found to be immune to a later intracerebral inoculation, manifesting only a rise in temperature.

Sheep are not susceptible to natural infection with the virus of vesicular stomatitis but the intracerebral inoculation with the New Jersey type resulted in the usual syndrome manifested in horses and cattle. Local infection with the virus was not established on the tongue of this species. After a local exposure to the New Jersey type virus, a sheep was not immune to a later intracerebral inoculation.

The intracerebral inoculation of horses, cattle, and sheep with the Indiana type virus was followed by a rise in temperature, and in two cases, a mare and colt developed more severe clinical symptoms, but all animals recovered.

The greater virulence of the New Jersey type of vesicular stomatitis virus in intracerebral injections in large animals was demonstrated in this investigation. This is in accord with previous research wherein the New Jersey type virus was shown to invade more quickly than the Indiana type.

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The Isolation of Actinomyces Bovis from Fistulous Withers and Poll Evil

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THE TWO DISEASES of the horse known as fistulous withers and poll evil may be described briefly as being chronic in nature, developing slowly over a period of time. There is usually a severe reaction of the affected tissues with swelling, suppuration, and the formation of a variable quantity of exudate. In the advanced cases of fistulous withers, it is not uncommon to find severe bone necrosis. The tissues may apparently heal following treatment only to have a discharge reoccur several months or a year later.

The nature of the lesions suggest the possibility of anaërobic organisms being involved. A search of the literature failed to reveal any extensive investigations of the anaërobic flora of clinical material; therefore, such an investigation was started. As a result, *Actinomyces bovis** has been isolated from 40 typical clinical cases studied during the past few years.

PROCEDURE

The material for this investigation was obtained from 50 cases of fistulous withers and 5 of poll evil presented at the Kansas State College veterinary clinic for treatment. When it seemed desirable that a more complete examination should be made, horses were purchased for autopsy. Only those cases that were typical, unopened, and had never received any treatment were used to obtain material for bacteriologic examination. In some horses, the infection had been present only a few weeks while in other cases the condition had been developing for months.

Collection of Material.—In collecting the material, aseptic precautions were observed to prevent its contamination. In the case of fistulous withers, the skin and underlying tissues were anesthetized. A liberal incision was made through the skin and tissues into the cavity and the accumulated exudate permitted to drain out. Experience has shown that it is difficult to isolate *A. bovis* from this material. Sterile hemostats were used to hold open the incision while a sterile curette was introduced into the region of the supraspinous bursa, where vigorous curettings were made from the tops of the supraspinous processes of the second, third, fourth, and fifth thoracic vertebrae and from the undersurface of the ligament in this same area. This material was placed in sterile Petri dishes and not more than three curettings to a dish. It has been found important to make a large number, 20 or more, of these scrapings in order to insure isolation of the organism. In cases where the operator was unable to definitely find a tract leading to the region of the supraspinous bursa, the isolation of the organism was difficult.

In the poll evil cases, the atlantal bursa was removed surgically and taken to the laboratory for culturing, pieces of the bursa wall and scrapings from the inner surfaces being used for culture material.

Culture Mediums.—Sugar-free meat infusion broth containing 3 per cent proteose peptone, 0.5 per cent salt, and adjusted to a pH of 7.4 was used as a base for preparing the following culture mediums used in this work: 1 per cent dextrose agar (1 per cent agar), Brewer's[1] sodium thioglycolate anaërobic broth, and 20 per cent sugar-free gelatin. Stock cultures were maintained in a 1 per cent sugar-free agar.

Technique of Isolation.—The material collected for examination was transferred into deep tubes of melted dextrose agar by means of small sterile spatulas. Because of the apparent nonuniform distribution of *A. bovis* in material collected, and difficulty encountered in homogeneously mixing this material, it was necessary to inoculate heavily (1 to 3 Gm.) 25 or more tubes in order to insure the obtaining of several tubes from which satisfactory isolation could be made. When pieces of necrotic ligament were planted in agar shakes, colonies of *A. bovis* were often observed growing out

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*Rosebury and coworkers prefer the species name *Actinomyces israeli* but because of the more general use of the name *Actinomyces bovis* it would seem preferable in a journal of this type.

Chart I—Biochemical Reaction of 40 Strains of A. bovis Isolated from Fistulous Withers and Poll Evil

Strains of *A. bovis*	Dextrose	Levulose	Galactose	Glycerine	Maltose	Lactose	Sucrose	Mannitol	Arabinose	Xylose	Dulcitol	Raffinose	Inulin	Salicin	Gelatin
Equine Origin															
1	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
2	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
3	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
4	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
5	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
6	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
7	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
8	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
9	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
10	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
11	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
12	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
13	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
14	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
15	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
16	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
17	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
18	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
19	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
20	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
21	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
22	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
23	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
24	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
25	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
26	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
27	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
28	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
29	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
30	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
31	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
32	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
33	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
34	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
35	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
36	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
37	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
38	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
39	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
40	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
Human Origin															
1	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+
2	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+

+ = Acid formation or proteolysis of gelatin. No gas formed by any strain.

from the surfaces of the ligament. All cultures were incubated thirty days before discarding, and examined at weekly intervals for growth. Typical colonies were picked by means of Pasteur pipettes, stained by Gram's method and, if organisms were found typical, transferred to Brewer's[1] sodium thioglycolate broth containing 1 per cent dextrose. After a heavy growth was obtained in this broth, transfers were made into sugar-free thioglycolate broth. This culture was used to inoculate 14 different fermentation mediums and sugar-free 20 per cent gelatin. It was found desirable to make use of a relatively large amount of inoculum in all transfers.

A marked improvement in the technique of isolation was attained during this particular study. Isolations were made from only 60 per cent of the 22 cases investigated in 1941-1942, whereas the organism was recovered from 82 per cent of the 33 cases studied in 1943-1944 and from 93 per cent of the 1944 cases.

Fermentations and Proteolysis.—Fermentation tubes were prepared by adding a sterile solution of the substrate to the sodium thioglycolate sugar-free base to give a 1 per cent concentration. The spot-plate method was used to determine acid formation since the addition of indicator to the mediums proved unsatisfactory. Mannitol fermented more slowly than any other substrate. All fermentation tests were incubated for fifteen days at which time the mannitol culture was tested for acid and if found negative, incubation was continued for an additional fifteen days. Gas production was determined by means of Durham fermentation tubes. No gas was formed by any strain.

A 20 per cent gelatin in the sugar-free base proved satisfactory for testing proteolytic action. The presence of fermentable sugar rendered the gelatin medium unsatisfactory, while the presence of 20 per cent gelatin facilitated anaërobic growth without the addition of sodium thioglycolate. Gelatin cultures were incubated at 37 C. for fifteen days; if proteolysis had not taken place, they were incubated an additional fifteen days. All strains liquefied gelatin.

DESCRIPTION OF ORGANISM

In general, the cultures of *A. bovis* isolated from fistulous withers and poll evil conformed to previous descriptions of the "smooth" type of this organism as described by Rosebury, Epps, and Clark[7] and Rosebury[6]. In view of certain modifications in the technique employed and also because all previous isolations were made from sources other than horses, it seems desirable to include a brief summary of morphologic and cultural characteristics.

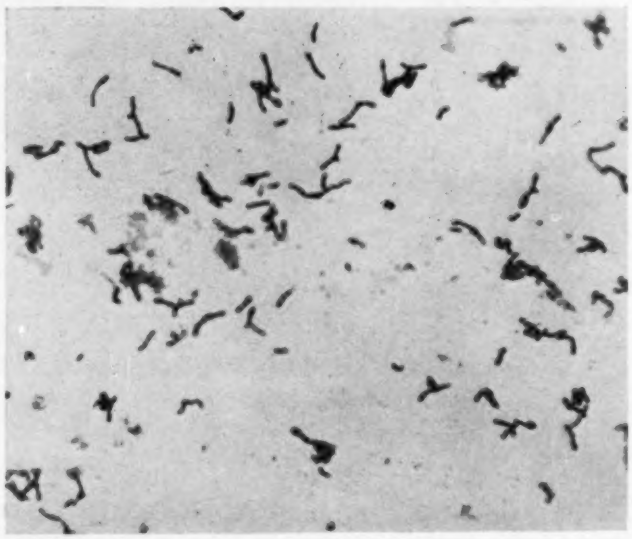

Fig. 1—Diphtheroid form of the "smooth" type of Actinomyces bovis from 4-day-old shake culture.

Staining and Morphology.—Pleomorphic diphtheroid microörganisms consisting of slender fusiform and filamentous elements (fig. 1). Cultures frequently assumed a short-rod form not characteristic of the species as originally described, this form being interchangeable with the diphtheroid. Occasionally, forms were observed with enlarged ends, but typical club formation was

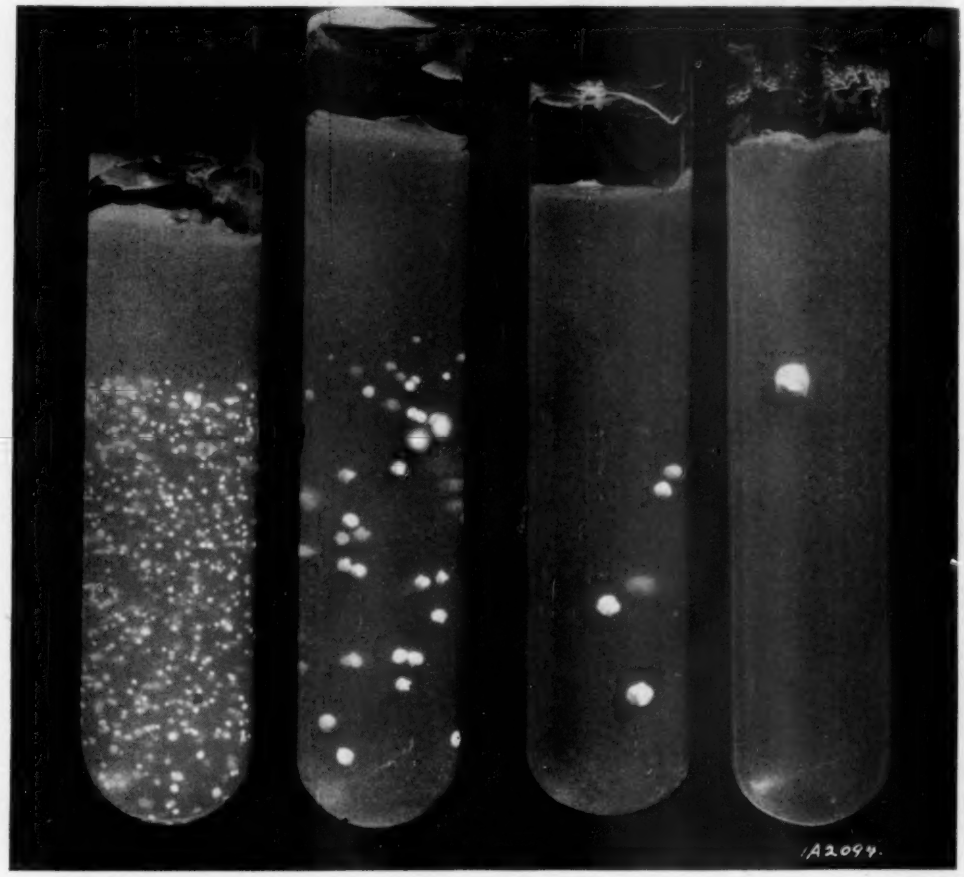

Fig. 2—Dextrose agar shake cultures of the "smooth" type of Actinomyces bovis, incubated twenty days.

not observed. Nonmotile, gram-positive, nonacid fast.

Cultural Features.—Obligate anaërobe, saccharophilic, and proteolytic.

Dextrose Agar Shakes. — No growth above 1 to 2 cm. (fig. 2); the "zone growth" so commonly described was observed occasionally. Colonies white, sometimes assuming a pinkish-brown color with age, soft, and with fin-like projections, (fig. 2); when sparsely seeded, attained a diameter of 4 to 8 mm. Retain viability two to four months in this medium; retain viability in dextrose-free agar six to eight months.

Brewer's[1] Sodium Thioglycolate Broth.—Very luxuriant fluffy growth which generally extends over half way up the column of broth. Will survive sixty days at room temperature.

Relationship to O_2.—No visible colonies by the burning candle method (10% CO_2). Good growth on dextrose agar plates under complete anaërobic conditions (O_2 absorbed by alkaline pyrogallic acid). Colonies soft, smooth, raised, moist, and opaque.

Hemolysis.—Slight; appearing in shake cultures containing a small per cent (0.25%) of blood, and on blood agar plates containing 1 per cent dextrose, incubated under anaërobic conditions.

Sugar Fermentation and Proteolysis.—(*see*, chart 1).

THE SIMILARITY BETWEEN A. BOVIS ISOLATED FROM FISTULOUS WITHERS AND FROM ACTINOMYCOSIS OF MAN

A survey of the literature dealing with the isolation of *A. bovis* from clinical mate-

rial leads to the conclusion that the organism must be quite variable in certain of its morphologic and cultural characteristics. Holm,[5] Emmons,[2] Vawter,[8] and others have described two types. Recently Rosebury, Epps, and Clark[7] and Rosebury[6] clarified the confused situation with respect to the types of this organism, by submitting conclusive evidence of the existence of "rough" and "smooth" variants. From the studies of Rosebury and coworkers it would appear that most of the previously recorded variations within this species can be attributed to normal variations within these two types. All 40 isolations from fistulous withers and poll evil made in this laboratory conformed to the "smooth" type described by Rosebury.

Recently, the writers had an opportunity to isolate *A. bovis* from 2 local cases of cervico-facial actinomycosis of man. In both instances, two distinct types of the organism were isolated. One of these types conformed morphologically and culturally with the strain isolated from fistulous withers; its fermentation and proteolytic characteristics are included in chart 1 for comparison. The other type conformed to the "rough" type described by Rosebury and differed from the equine strains primarily in the following respects: cells more elongated; greater tendency to branch; less tendency to break up into unicellular elements; no clouding of broth; colony more firm and rough with a "cauliflower-like" growth; no liquefaction of gelatin; inconstant fermentation reaction; does not maintain its viability nearly as long in most laboratory mediums.

From the recorded descriptions of *A. bovis*, it is evident that a large majority of the types previously studied have been "rough." This accounts for the widespread conception of *A. bovis* existing only as a distinctively branching, filamentous organism.

DISCUSSION

For several years the writers have been investigating the presence of anaërobic microörganisms in the clinical conditions of the horse known as fistulous withers and poll evil. Thus far, a single anaërobe has been isolated from 40 of 55 typical cases. This organism has been identified, both in this and other laboratories, as *A. bovis*, the recognized cause of the widespread condition found particularly in man and cattle known as actinomycosis.

The exudate from fistulous withers differs from that of actinomycosis in one significant respect, *i. e.*, it does not contain the club-bearing rosettes or "sulfur granules". The presence of "sulfur granules," however, is not an essential characteristic of actinomycotic exudate. *A. bovis* has been isolated from clinical cases of human actinomycosis in which no rosettes could be demonstrated. In view of the fact that "sulfur granules" have never been encountered in clinical material from fistulous withers and that only the "smooth" form of the organism has been isolated from such material, it is suggested that rosette formation may be associated only with the "rough" type. Furthermore, these structures are by no means specific for *A. bovis*, having been recorded for a number of other infections, and even produced by injecting nonliving material. For a discussion of this subject reference is made to publications by Hoeppli[4] and Emmons[3].

The fact that *A. bovis* has never been isolated from material extraneous to the animal body, the frequency with which it has been found in fistulous withers, and the large number of colonies frequently developing from clinical material furnish strong presumptive evidence of growth of the organism within the involved tissues.

SUMMARY

Actinomyces bovis has been isolated from 40 of 55 typical previously unopened cases of fistulous withers and poll evil. So far as the writers are aware this is the first reported association of *A. bovis* with these diseases.

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Prognostic Value of Blood Determinations in Certain Surgical Conditions of the Horse

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SINCE it has long been known that blood determinations are of considerable aid in diagnosis, an attempt here has been made to evaluate them for prognostic purposes. Many physicians are routinely using blood determinations for prognosis in surgical conditions and they report them to be of value. It seems probable that such procedures would also have some significance to the veterinarian in foretelling the outcome of surgical conditions in large animals.

The material for this study included equine patients entered in the Iowa State College Veterinary clinic for treatment. Inasmuch as these horses were not a selected group, were of different size, sex, age, and breed, and the fact that they had previously been subjected to such variables as differences in nutrition, care, and work, puts limitations on the investigation. In other words, the study was clinical rather than controlled research.

The principal surgical conditions observed were: supraspinous bursitis commonly called "fistula of the withers" and necrosis of the lateral cartilage of the third phalanx, more commonly known as "quittor." For purposes of comparison, a series of eight blood determinations were made on six apparently normal horses. Only one of these charts will be represented in this paper.

METHOD OF PROCEDURE

A) Method of Obtaining Blood.—The most successful method for obtaining blood consisted of puncturing the jugular vein with a sharp, sterile, 20-gauge hypodermic needle. Repeated venipunctures were at first feared because of the possibility of phlebitis. This did not occur. In one patient, the jugular veins were punctured 39 times over a period of fifty-five days without showing significant damage.

No clipping or shaving of the hair over the jugular vein was necessary. The area for venipuncture was prepared by freely rubbing the skin and hair with ethyl ether applied on cotton, followed by 70 per cent ethyl alcohol.

The initial punctures were made about 2 inches posterior to the external maxillary vein; the succeeding punctures were each a little more posterior and, in addition, the two jugular veins were used alternately. The vein was raised by compressing with the thumb back of the site of puncture.

B) Making Determinations and Counts.—Blood for the erythrocyte and leucocyte counts, the differential count, and the hemoglobin determination was used without an anticoagulant and immediately after withdrawal. Samples were obtained from patients tied in their stalls or confined in stocks.

The blood as it came from the needle was received in the depression of the hollow-ground slide; three or four drops were sufficient. The blood smears, the cell count dilutions, and hemoglobin preparation were made beside the animal. It should be emphasized that these procedures should be done with as little loss of time as possible, lest the blood cells settle out or a clot form. A 24-gauge wire loop, as used in inoculating bacteria on mediums, was employed to transfer a drop of blood to the dry, clean, fat-free glass slides. The drop was then evenly and smoothly spread by the narrower edge of a second slide held at an angle of approximately 45 degrees. A few seconds of rapidly waving the smear in the air insured quick drying of the blood. Two smears were made for each determination. Next, the leucocyte dilution was made, then the dilution for the erythrocytes, and lastly the dilution of blood with 1 per cent hydrochloric acid in the Newcomer pipette for hemoglobin determination.

All material was then taken to the laboratory where the blood cells were counted, the hemoglobin was measured, and the blood smears were stained.

The erythrocyte and leucocyte counts were made on a double Neubauer "Bright-Line" counting chamber.

An abridgement of the thesis presented to the faculty of Iowa State College in fulfillment of the requirement for the degree of Master of Science. (The thesis had many more hemograms than are herein represented.)

The hemoglobin determinations were made on a Newcomer hemoglobinometer.

The most difficult and important part of this study had to do with preparing and reading the smears for differential leucocyte counting. It is to be emphasized that a thin, even, blood smear must be obtained; that the smear be clearly stained, and that the different leucocyte types be carefully studied and properly identified.

In examining the differential smear, the leucocytes were classified under the headings: neutrophiles, basophiles, eosinophiles (granulocytes), monocytes, and lymphocytes. The detailed differentiation made on the group of cells classed as neutrophiles was the Pons and Krumbhaar[1] modification of the Schilling[2] classification as follows:

1) The Juvenile Neutrophiles: These are very young forms in which the nucleus is slightly indented. To belong in this class, the nucleus should not be notched more than fifty per cent of its diameter.

2) The Stab Cells or Nonsegmented Neutrophiles: For this class the nucleus is narrow, cylindrical, and bent like the letters or numbers: U, V, W, T, S, F, E, 6, 8, 3.

3) The Segmented Neutrophiles: In these the nucleus is divided into from two to five distinct segments showing no visible connections or at most a union of thin threads of chromatin.

As a rule, a total of 100 leucocytes were classified for each determination. Only in cases where the leucocyte count was around 30,000 to 40,000 were 200 or 300 cells classified.

C) Method of Recording: the Hemogram.—The hemogram is a very convenient and inclusive chart for recording the blood determinations and plotting the Schilling index. A modification of Schilling's[2] method of constructing hemograms was used; the "shiftograph" idea was adopted from Crocker and Valentine.[3] The "shiftograph" is the red line used to follow various shifts to the left or right as based on the Schilling index. In other words, it is a graphic presentation of the Schilling index.

The Schilling index is the number resulting from the division of the stab neutrophiles plus the juvenile neutrophiles by the segmented neutrophiles. It represents the ratio of immature to mature neutrophiles. A Schilling index over one (1.0) would indicate that there were more immature than mature neutrophiles.

Hemogram I—"Normal Horse" Case A—An 11-Year-Old Mare

D	Hb	WBC	RBC	Sch I	J	St	Se	TN	B	E	M	L		Remarks
	Shiftograph			9	8	7	6	5	4	3	2	1	0	
1	12.5	9280	11.5	0.3	0	13	43	56	2	1	0	41		May 21
3	12.5	9760	8.5	0.2	0	12	66	78	0	2	0	20		" 23
5	12.5	10440	9.7	0.2	0	13	60	73	0	0	0	27		" 25
7	12.0	10000	9.2	0.2	0	12	55	67	1	1	1	30		" 27
9	12.0	10680	9.5	0.2	0	11	64	75	0	1	0	24	*	" 29
11	13.0	12080	8.6	0.1	0	5	47	52	0	7	0	41		" 31
13	12.8	9560	9.8	0.1	0	5	53	58	0	3	0	39		June 2
21	12.9	10320	9.9	0.1	0	6	43	49	0	4	1	46		" 10

*Encephalomyelitis vaccination.

D = day of examination; Hb = hemoglobin in Gm. per 100 cc. of blood; WBC = leucocyte count per cmm. of blood; RBC = erythrocyte count per cmm. of blood in millions; Sch. I = Schilling index; J = percentage of juvenile neutrophiles; St = percentage of stab neutrophiles; Se = percentage of segmented neutrophiles; TN = percentage of total neutrophiles; B = percentage of basophiles; E = percentage of eosinophiles; M = percentage of monocytes; L = percentage of lymphocytes.
Red line horizontally intersecting "shiftograph" indicates surgical operation.
The line below the symbols carries the numbers for the "shiftograph."

Case A.—A hemogram run on a normal horse follows. This horse was an 11-year-old white Percheron mare.

QUITTORS

A "quittor" refers to an infection and necrosis of the lateral cartilage or cartilages of the third phalanx. This condition can result from any wound involving the cartilage; the most common predisposing factor is a fence-wire laceration.

Several operations are described for such a condition; however, the most satisfactory technique used in the Iowa State College Veterinary Clinic is briefly as follows: A semilunar incision is made through the skin and subcutis over the free border of the cartilage and posteriorly over the heel. Another incision is made one-half to one inch above the coronary border of the hoof. This semicircular portion of skin is then removed. The tissue below is cut or curetted out until all necrotic cartilage has been removed.

At least one, and if possible, two or three

blood determinations are obtained on every patient prior to the operation.

Following are some of the hemograms made from horses operated on for "quittor."

Hemogram 2—"Quittor" Case B

D	Hb	WBC	RBC	Sch I	J	St	Se	TN	B	E	M	L	Remarks	
	Shiftograph		9	8	7	6	5	4	3	2	1	0		
1	9.2	13560	9.26	2.2	0	52	23	75	0	3	2	20		Apr. 28
2	8.8	10240	7.92	2.3	2	40	17	59	0	5	2	34	100.8°F.	" 29
4	9.0	11800	7.00	2.2	0	48	22	70	0	6	5	22	101.0°F.	May 1
6	8.2	11960	6.66	3.0	0	54	18	72	0	6	0	22	101.0°F.*	" 3
7	9.5	13600	8.82	3.6	0	50	14	64	2	6	4	24	101.4°F.	" 4
9	9.2	12320	7.44	4.2	0	47	11	58	0	6	4	32	101.4°F.	" 6
11	9.9	12400	9.00	2.7	0	43	16	59	1	3	1	36	101.6°F.	" 8
13	8.5	13680	8.04	2.1	0	48	22	70	0	3	0	27	100.5°F.	" 10
15	9.2	11160	8.20	1.3	0	34	26	60	0	6	4	31	101.0°F.**	" 12
						Discharged								

*Operation on May 2; **Wound granulating nicely.

D = day of examination; Hb = hemoglobin in Gm. per 100 cc. of blood; WBC = leucocyte count per cmm. of blood; RBC = erythrocyte count per cmm. of blood in millions; Sch. I = Schilling index; J = percentage of juvenile neutrophiles; St = percentage of stab neutrophiles; Se = percentage of segmented neutrophiles; TN = percentage of total neutrophiles; B = percentage of basophiles; E = percentage of eosinophiles; M = percentage of monocytes; L = percentage of lymphocytes.
Red line horizontally intersecting "shiftograph" indicates surgical operation.
The line below the symbols carries the numbers for the "shiftograph."

Case B.—On April 28, 1942, a 6-year-old brown mare of medium draft type entered the clinic with a "quittor" on one front foot.

Three preoperative counts were made on this patient. It is readily seen that the blood picture was abnormal as evidenced by the Schilling index above two (2.0). The regularity of the index for the three days prior to operation would indicate that the condition was not readily changing. On the sixth day, the first post-operative count was made. Already the index had started to shift to the left until the climax was reached on the ninth day. From that day on, the blood picture changed; the Schilling index began to shift to the right. This course is indicative of a favorable progress.

The fact that the total leucocyte count was by no means high would indicate one of two things: either that the wound was relatively free from invasion by virulent bacteria and had good drainage remaining so throughout the course of the condition, or that the patient's hematopoietic tissues failed to respond to the demand necessary to bring about repair or combat infection. This latter, however, is highly improbable in view of the fact that the Schilling index did not go alarmingly high, nor did it fail to return to somewhere near the normal figure. Ten days following the operation (May 13, 1942), the patient was discharged from the clinic, at which time she exhibited very little lameness and the wound had started to heal nicely.

Case C.—This "quittor" case was an 11-year-old black mare that had injured her foot four months previously on smooth wire. She was quite lame.

The first three counts prior to the operation, with a Schilling index almost two (2.0) and a very slightly elevated leucocyte count, would indicate a mild type of disease. As usually happens following the first operation, a temporary slight rise in the Schilling index took place.

Clinically, the patient showed improvement until the tenth day. The body temperature remained within normal limits and the total leucocyte count gave no indication of what was occurring in the blood. From all clinical appearances, the patient could have been sent home and regarded as well on the road to recovery, but the Schilling index indicated otherwise. On the ninth day it had materially shifted to the left and continued to do so for another day. Thus the Schilling index antedated the clinical symptoms, the temperature reaction and the leucocyte count by two days. Something happened on the eighth day that changed the course and prognosis of the case. Either

the joint capsule had been opened on the day of the operation and infection became established five days later or else a piece of necrotic cartilage or adjacent tissue had started to act as a severe irritant.

ently this afforded partial relief as evidenced by the shift to the right, but only of a temporary nature since the "shiftograph" again returned to the left on the sixteenth day followed by an increase in

Hemogram 3—"Quittor" Case C

D	Hb	WBC	RBC	Sch I	J	St	Se	TN	B	E	M	L	Remarks	
	Shiftograph		9	8	7	6	5	4	3	2	1	0		
1	11.2	14500	11.5	1.0	0	36	36	72	0	1.0	1.0	27	100.6°F.	Apr. 1
2	9.5	12880	8.6	2.0	0	51	21	72	2	0.0	1.0	26	100.8°F.	" 2
3	8.5	14800	7.4	1.6	0	50	31	81	0	0.0	1.0	17	100.8°F.	" 3
5	9.0	14520	7.3	2.1	0	52	19	71	2	0.0	2.0	25	101.0°F.	" 5
6	9.5	12000	9.0	2.5	0	49	19	68	0	3.0	1.0	28	101.0°F.	" 6
7	9.3	12120	7.7	1.7	0	49	28	77	2	2.0	2.0	18	100.8°F.	" 7
8	8.2	13440	6.8	1.6	0	45	29	74	1	1.0	1.0	22	101.6°F.	" 8
9	8.7	14640	8.9	3.0	0	59	19	78	2	0.0	1.0	19	101.0°F.	" 9
10	9.0	13360	7.7	4.4	1	60	13	74	0	1.0	2.0	23	100.8°F.*	" 10
12	9.2	19720	7.8	4.2	0	70	17	87	1	0.0	0.0	12	102.4°F.	" 12
13	11.2	17000	7.9	2.5	0	63	25	88	1	0.0	3.0	8	103.2°F.	" 13
14	13.0	19080	9.5	2.2	0	59	27	86	2	0.0	1.0	11	102.0°F.	" 14
15	14.0	16480	9.3	2.7	2	60	22	84	0	0.0	2.0	15	102.2°F.	" 15
16	13.5	16000	7.9	4.5	0	68	15	83	1	0.0	1.0	15	103.2°F.**	" 16
17	14.0	21160	11.0	4.3	0	72	17	89	0	0.0	2.0	9	104.4°F.	" 17
18	12.5	20360	9.0	4.0	4	71	19	94	0	0.0	0.0	7	104.4°F.	" 18
20	12.7	17660	10.0	2.2	2	50	22	74	0	0.5	0.5	24	103.4°F.	" 20
21	14.8	19560	9.5	3.5	0	62	18	80	0	2.0	2.0	16	103.2°F.	" 21
22	13.5	19000	8.6	4.0	0	74	18	92	0	1.0	3.0	4	102.5°F.	" 22
23	12.2	22480	9.1	3.2	0	62	19	81	0	0.0	2.0	18	103.9°F.	" 23
24	12.5	20720	8.8	4.4	0	67	15	82	1	1	2	14	102.5°F.	Apr. 24
25	13.5	22200	9.3	3.0	0	60	19	79	1	0	3	17	103.0°F.	" 25
27	12.0	16400	8.2	3.3	0	57	17	74	0	0	1	25	103.6°F.***	" 27
29	14.0	20000	8.9	3.0	0	60	21	81	0	0	2	18	103.0°F.***	" 29
30	12.4	24640	10.1	3.3	0	63	19	82	0	0	0	18	103.2°F.***	" 30
31	13.5	26250	8.4	4.6	0	67	14	81	0	0	0	19	103.2°F.***	May 1
32	13.5	25360	8.5	4.2	0	72	17	89	0	0	1	10	102.6°F.***	" 2
34	10.5	21280	8.4	3.2	0	58	18	76	2	0	3	19	103.4°F.***	" 4
35	9.5	23840	8.5	5.4	0	71	13	84	0	2	2	12	103.0°F.***	" 5
36	9.5	23320	7.8	4.2	0	72	17	89	0	0	0	11	102.4°F.	" 6
37	7.5	22720	6.0	4.0	0	60	20	80	0	0	0	20	102.6°F.***	" 7
39	8.0	27080	7.5	7.5	0	75	10	85	1	0	1	14	104.2°F.***	" 9
40	9.2	27520	6.3	6.6	0	78	9	87	0	0	1	13	102.6°F.***	" 10
42	9.0	25200	6.2	6.0	0	71	13	84	0	0	1	15	103.6°F.***	" 12
43	8.2	25680	5.5	6.0	0	74	12	86	1	0	0	13	103.6°F.***	" 13
45	8.9	27280	8.0	6.0	0	69	12	81	0	0	0	19	102.6°F.	" 15
49	7.5	16860	6.0	3.0	0	57	20	77	0	1	0	23	101.8°F.	" 19
52	7.0	18240	5.2	4.0	3	69	18	90	0	0	2	8	102.0°F.	" 22
55	6.8	19920	6.1	2.2	2	54	25	81	0	0	0	19	102.0°F.	" 25
							Destroyed							

*Clinical improvement ceased; **Exhibiting severe pain; ***1 oz.. of sulfanimide given.

D = day of examination; Hb = hemoglobin in Gm. per 100 cc. of blood; WBC = leucocyte count per cmm. of blood; RBC = erythrocyte count per cmm. of blood in millions; Sch. I = Schilling index; J = percentage of juvenile neutrophiles; St = percentage of stab neutrophiles; Se = percentage of segmented neutrophiles; TN = percentage of total neutrophiles; B = percentage of basophiles; E = percentage of eosinophiles; M = percentage of monocytes; L = percentage of lymphocytes.
Red line horizontally intersecting "shiftograph" indicates surgical operation.
The line below the symbols carries the numbers for the "shiftograph."

This illustrates the limitations of a leucocyte and differential count without determining the Schilling index.

A second operation was performed on the twelfth day at which time additional necrotic cartilage was curetted out. Apparleucocytes on the seventeenth day, either indicating another focus of infection or the presence of some other irritant. No gross indications for a third operation could be found and it looked as though the patient would overcome the infection as the leuco-

cyte count and Schilling index decreased. Such was not the case and the third operation, which perhaps should have taken place on the sixteenth or seventeenth day, was postponed until the twenty-first day. By this time, the entire coronary region above the hoof had become markedly swollen and reparative processes, the "shiftograph" turned to the right. After another superficial abscess was opened on the fifty-first day, the Schilling index returned to less alarming ranges.

Clinically, the patient showed little improvement during the last week. Her condition had been considered incurable after three or four weeks care but further treatment was carried out at the owner's request. Permission was finally obtained for her destruction.

Hemogram 4—"Fistula of the Withers" Case D

D	Hb	WBC	RBC	Sch I	J	St	Se	TN	B	E	M	L	Remarks	
	Shiftograph			9	8	7	6	5	4	3	2	1	0	
1	9.0	16280	8.15	7.0	0	40	7	56	0	8	1	36	100.6°F.*	July 14
2	8.0	20520	7.70	7.7	0	46	6	52	0	1	1	46	101.8°F.	" 15
3	7.0	15080	7.15	7.7	0	46	6	52	0	3	1	44	101.8°F.	" 16
5	8.5	12640	8.00	5.2	0	42	8	50	1	3	0	46	102.0°F.	" 18
7	8.5	12960	8.40	4.8	0	42	10	52	1	4	0	43	101.5°F.	" 20
9	7.8	13880	7.45	3.0	0	38	13	51	0	1	0	48	100.6°F.	" 22
11	7.0	16000	7.20	3.8	0	49	13	62	1	3	3	32	100.2°F.	" 24
13	8.2	18720	9.00	5.7	0	57	10	67	0	7	0	26	100.0°F.	" 26
15	9.0	17400	7.50	3.7	0	52	14	64	1	4	1	28	99.8°F.	" 28
17	9.5	18880	9.00	4.2	0	55	13	68	1	5	1	26	99.8°F.	" 30
21	8.7	15600	10.00	4.5	0	54	12	66	0	2	4	28	100.5°F.	Aug. 3
23	10.0	12000	9.00	2.8	0	48	17	65	0	1	1	33	100.5°F.	" 5
26	10.5	14840	8.50	2.6	0	42	16	58	0	6	1	35	100.0°F.	" 8
28	10.5	11000	7.00	2.2	0	41	18	59	0	4	1	36	99.5°F.	" 10
							Discharged							

*These counts were made prior to the operation.

D = day of examination; Hb = hemoglobin in Gm. per 100 cc. of blood; WBC = leucocyte count per cmm. of blood; RBC = erythrocyte count per cmm. of blood in millions; Sch. I = Schilling index; J = percentage of juvenile neutrophiles; St = percentage of stab neutrophiles; Se = percentage of segmented neutrophiles; TN = percentage of total neutrophiles; B = percentage of basophiles; E = percentage of eosinophiles; M = percentage of monocytes; L = percentage of lymphocytes.

Red line horizontally intersecting "shiftograph" indicates surgical operation.

The line below the symbols carries the numbers for the "shiftograph."

it was necessary to open an abscess pocket anteriorly. Good drainage was provided and the foot was again dressed and bandaged. Other foci of infection apparently still existed as evidenced by a persistent, and later gradually increasing, Schilling index accompanied by a high leucocyte count.

On the thirty-fifth day, the severity of the infection had reached another peak and by this time it had spread to the cartilage on the opposite side of the foot. An abscess was surgically drained through this cartilage but apparently other foci of infection still existed because after a short shift to the right, the index continued to go to the left.

On the fortieth day, another abscess pocket was opened. This operation must have provided adequate drainage because, although the leucocyte count remained elevated probably to carry out the necessary

Necropsy revealed that the horse had been suffering from a suppurative arthritis which had begun to heal with phalangeal ankylosis.

Besides keeping the foot clean, dressed, and bandaged during the treatment, sulfanilamide was given *per orum* to help combat the infection. This is a probable explanation for the drop in hemoglobin concentration from its one time high of 14.8 to 6.8 mg. per cent.

FISTULA OF THE WITHERS

"Fistula of the Withers" is a condition very commonly found in Iowa horses. Its origin is usually in the supraspinous bursa located over the spines of the second to the

Hemogram 5—"Fistula of the Withers" Case E

D	Hb	WBC	RBC	Sch I	J	St	Se	TN	B	E	M	L	Remarks	
	Shiftograph			9	8	7	6	5	4	3	2	1	0	
4	11.0	15120	7.63	0.8	0.0	39	45	84	1.0	0	1.0	14	102.2°F.	Nov. 24
11	11.5	8220	7.45	0.7	0.0	37	50	87	1.0	0	1.0	11	102.8°F.	" 29
14	14.0	20660	9.00	3.5	0.5	70	20	90	0.5	0	0.5	8	102.4°F.	Dec. 1
15	13.0	25280	9.44	6.2	3.0	75	12	91	0.0	0	0.0	9	102.0°F.	" 3
16	9.0	20600	8.34	7.8	0.0	78	10	88	0.5	0	0.5	11	102.6°F.	" 4
							Destroyed							

D = day of examination; Hb = hemoglobin in Gm. per 100 cc. of blood; WBC = leucocyte count per cmm. of blood; RBC = erythrocyte count per cmm. of blood in millions; Sch. I = Schilling index; J = percentage of juvenile neutrophiles; St = percentage of stab neutrophiles; Se = percentage of segmented neutrophiles; TN = percentage of total neutrophiles; B = percentage of basophiles; E = percentage of eosinophiles; M = percentage of monocytes; L = percentage of lymphocytes.
Red line horizontally intersecting "shiftograph" indicates surgical operation.
The line below the symbols carries the numbers for the "shiftograph."

fourth or fifth thoracic vertebrae. For some reason, not yet known, this bursa shows inflammation characterized by considerable serous exudate. Not until the bursa becomes greatly distended, finally causing a bulge on one or both sides of the withers, are there any clinical symptoms. Later an abscess forms and the adjacent portion of the ligamentum nuchae becomes necrotic. The complicated arrangement of muscles in this region allows many possible locations for further abscess formation.

Many times, large pieces of necrotic ligamentum nuchae are removed, as are also the tops of any necrotic vertebral spines.

In "fistula of the withers" cases, frequently secondary abscesses form, usually deeper than the first one; these are always opened and drained surgically. An attempt is made to open and drain all abscess pockets that form before the patient is sent home. Often as many as four or five operations are necessary to open all abscesses and remove all necrotic tissue.

Hemogram 6—"Fistula of the Withers" Case F

D	Hb	WBC	RBC	Sch I	J	St	Se	TN	B	E	M	L	Remarks	
	Shiftograph			9	8	7	6	5	4	3	2	1	0	
1	8.5	13660	5.23	3.2	0	64	19	83	0	1	1	15	101.8°F.	Apr. 3
2	9.9	11480	7.24	2.2	0	53	24	77	0	3	2	18	102.4°F.	" 4
4	8.5	12240	7.01	2.0	1	53	26	80	0	0	1	19	102.4°F.	" 6
5	8.7	13280	6.32	1.9	3	48	27	78	0	2	0	21	101.8°F.	" 7
6	8.2	10440	6.26	3.5	4	55	16	75	0	1	0	24	101.8°F.	" 8
7	9.0	10960	8.59	5.5	0	60	11	71	0	2	0	27	102.5°F.	" 9
8	8.2	9840	6.00	3.0	0	47	18	65	0	3	2	30	102.6°F.	" 10
10	7.9	8800	7.87	5.0	0	61	13	74	1	0	2	23	102.6°F.	" 12
11	7.5	9120	6.22	4.0	3	43	11	54	0	4	1	38	103.2°F.	" 13
12	7.8	11040	7.73	4.7	0	56	12	68	1	0	3	28	102.0°F.	" 14
13	9.8	8920	7.09	2.2	0	55	25	80	0	0	1	19	101.8°F.	" 15
14	8.2	11480	7.49	3.2	3	58	17	78	1	1	0	20	102.2°F.	" 16
15	8.8	12680	6.50	3.2	2	58	18	78	0	0	5	17		" 17
17	9.5	14080	7.76	3.8	1	58	15	74	0	1	4	22	100.6°F.	" 19
18	8.9	13180	7.62	2.5	0	53	20	73	0	1	1	25	100.4°F.	" 20
19	8.2	16000	6.43	2.2	0	50	22	72	0	1	1	26	100.6°F.*	" 21
20	9.5	14200	6.49	2.1	0	50	23	73	1	0	4	22	100.6°F.	" 22
							Discharged							

*Small abscess surgically opened.
Key to abbreviations for Hemogram 5 also applies to Hemogram 6.

Briefly, the operation consists of opening the enlargement on both sides of the withers and providing bottom drainage for the pockets of serous or purulent material.

Case D.—This was a 4-year-old black mare of draft type, with a 4-week-old suckling colt.

On entrance, she had a high Schilling

index but also a fairly high leucocyte count which would indicate that the bone marrow was active and still able to produce cells although many of them were not mature. A slight shift to the left and a rise in the leucocyte count was noticed following the operation on the first day. Then the leucocyte count dropped quite rapidly, perhaps too rapidly to combat the pathologic process because a reverse occurred starting on the ninth day and the "shiftograph" again went to the left. Nevertheless, the bone marrow responded and produced more cells which apparently carried the reparative process through successfully. The leucocyte count decreased as the Schilling index went to the right.

This hemogram would indicate that the patient had a responsive and adequate defensive mechanism through the medium of the blood cells and that she should be able to cope favorably with the surgical condition.

Case E.—The patient was a bay mare with large, nonfluctuating swellings on both sides of the withers. She entered the clinic Nov. 20, 1941; the first operation was on the second day. The first observation of the blood was not made until the fourth day, at which time it was noticed that the patient was depressed.

The first operation, which was performed before blood counts were made, revealed no abscess pockets. After extensive exploration nothing but fibrous inflammatory tissue was found. A second operation was performed on the eleventh day. During the seven intervening days between the first and second operations, the patient showed no improvement nor did she show any alarming symptoms other than extreme sensitivity over the region of the withers. At the second operation, large areas of necrotic ligamentum nuchae were removed along with necrotic portions of the vertebral spinous processes. From that day on, the patient regressed.

The hemogram shows the blood picture of a condition ending unfavorably. The two counts prior to the second operation showed a relatively insignificant Schilling index, in fact, not as high as would be expected from a patient recovering from an operation seven days previously, although the operation was not extensive. However, the high percentage of total neutrophiles suggested an abnormality. The first count after the second operation showed a marked shift to the left with an increase in the leucocyte count. On the next day, more leucocytes made their appearance, but still the Schilling index continued to climb. By this time it became advisable to destroy the horse. She was extremely depressed and unable to get up. Extensive subcutaneous edema and emphysema were present on the top line from the withers back to the sacral region. Apparently a gas gangrene infection had been introduced at or following the second operation.

Hemogram 7—"Strangles" Case G

D	Hb	WBC	RBC	Sch I	J	St	Se	TN	B	E	M	L	Remarks	
	Shiftograph			9 8	7	6	5	4	3	2	1	0		
1	10.5	15000	10.89	1.7	0	55	32	87	0	0	0	13	104.0°F.	May 5
2	9.9	24400	8.58	5.3	0	53	10	63	0	1	0	36	106.0°F.*	" 6
3	9.5	14680	8.86	5.3	0	59	11	70	0	5	1	24	99.8°F.	" 7
4	11.0	13720	8.40	3.0	0	57	19	76	0	1	1	22	99.8°F.	" 8
5	9.6	11760	7.31	1.5	0	45	31	76	0	3	2	19	102.0°F.	" 9
7	11.0	16080	8.63	1.5	0	50	32	82	0	0	2	16	101.4°F.	" 11
							Discharged							

*Absces surgically drained.

D = day of examination; Hb = hemoglobin in Gm. per 100 cc. of blood; WBC = leucocyte count per cmm. of blood; RBC = erythrocyte count per cmm. of blood in millions; Sch. I = Schilling index; J = percentage of juvenile neutrophiles; St = percentage of stab neutrophiles; Se = percentage of segmented neutrophiles; TN = percentage of total neutrophiles; B = percentage of basophiles; E = percentage of eosinophiles; M = percentage of monocytes; L = percentage of lymphocytes.
Red line horizontally intersecting "shiftograph" indicates surgical operation.
The line below the symbols carries the numbers for the "shiftograph."

Case F.—This "fistula of the withers" patient was a 12-year-old black mare with a 5-week-old suckling colt.

The hemogram showed that there was a decrease in leucocytes and Schilling index following the operation on the second day. On the sixth day conditions changed. The Schilling index shifted to the left but the leucocyte count decreased. Some irritant was acting, however; the hematopoietic system failed to respond. A minor operation the ninth day in which pieces of superficial necrotic tissue were removed, started the "shiftograph" on a fluctuating course to the right. Apparently the necrosed material had been surgically removed but not enough leucocytes were present to combat the existing infection until the seventeenth day. At that time, more leucocytes appeared and the Schilling index made a steady shift to the right. On the nineteenth day a small superficial abscess was opened. This did not affect the favorable course of the Schilling index.

STRANGLES

Case G.—This was a grey 5-year-old mare of saddle type. She had just been purchased at an auction ring and was entered in the clinic to be treated for strangles. The operation consisted of opening an abscess that had formed in the mandibular lymph nodes.

The chart shows what occurred to the blood picture during the development of the abscess, although not from the beginning, and also what occurred after surgical drainage had been established.

The abscess was opened on the second day, at which time the leucocyte count had risen to 24,000. This count decreased to 14,600 the day following the operation. Although the Schilling index was slower in descending, it started to the right on the fourth day and did not again shift to the left.

DISCUSSION

The greatest value of blood determinations for prognostic purposes lies in the total leucocyte count and the qualitative changes in the neutrophilic leucocytes. The fallacy of using a total leucocyte count and an ordinary differential, in which the neutrophiles are not separated into age groups, in foretelling the progress and outcome of a pathologic condition was pointed out in case C. The reality can only be pictured by a Schilling index.

The basis upon which so much value is given the Schilling index as an aid to prognosis is as follows: normally, in horses' blood, as is shown by the work on apparently normal horses, the stab cell neutrophile count ranges from 5 to 24 per cent of the total leucocytes. According to Hikmet,[4] these percentages are high, nevertheless the percentage of segmented neutrophiles was such that the Schilling index ranged from 0.1 to 0.4 and in one instance to 0.5 but never higher. When the Schilling index goes higher than these values, it is assumed that a strain is put on the patient, such as could come from an infection or other irritation, resulting in an outpouring of leucocytes at such a rate that maturation cannot keep pace with the demand and the young cells appear in increased number in the peripheral blood. Furthermore, it is assumed that the more severe the pathologic condition, the higher the per cent of young forms, that is to say, the higher the Schilling index.

In those cases in which the hemogram indicated a favorable prognosis, it was found that the blood counts responded in one of two ways: Either the leucocyte count increased and then the Schilling index decreased, or else the leucocyte count remained relatively stable while at the same time the Schilling index decreased. In either instance, the result was the same, a lowering of the Schilling index which would be a shift to the right.

It is upon the rightward trend (shift to the right) that a favorable outcome is based. Of course, an absolutely favorable outcome cannot be made unless the Schilling index continues its decrease and only then when it has reached normal limits, but certain trends can be ascertained before the patient completely recovers or dies.

It is evident that a hemogram as represented in case E is unfavorable. In case E the leucocytes violently responded on the

second and third day but were unable to turn the tide; the Schilling index continued to rise. On the fourth day, leucocytes decreased but the index continued to climb.

Although the index for case C made several encouraging shifts to the right, the general trend of the "shiftograph" was definitely unfavorable until after the fortieth day. From that time on, the horse's foot did start to heal with ankylosis of the infected joint and the index went to the right, but a horse with a stiff joint in that location would be useless, so she was destroyed.

Much can be learned from a study of case G (strangles). Here it is seen how the leucocyte count and Schilling index react when abscess formation is going on and what they do after surgical drainage has been provided. Similar changes can be seen on the charts for "fistula of the withers" and "quittor" cases, although not in such an outstanding way.

SUMMARY AND CONCLUSIONS

Erythrocyte and detailed leucocyte counts and hemoglobin measurements were conducted on certain surgical conditions in equine patients hospitalized at the Iowa State College Veterinary clinic, with the hope of gaining prognostic information. These determinations were recorded on charts (a modification of Schilling's hemogram) employing the "shiftograph" as a convenient method of graphically representing the Schilling index. With the graph, shifts to the right or left are readily seen.

From the blood findings, it was found that prognoses could be obtained, but only after a series of quite frequent blood counts had been made. Of most value for prognostic purposes was the Schilling index in conjunction with the total leucocyte count. Quite definite trends were established for those cases terminating favorably and those terminating unfavorably.

The following general conclusions may be made regarding the surgical conditions studied in the horse:

1) Blood determinations, as carried out in this investigation, reflect the presence and intensity of the pathologic processes studied and, when made at relatively frequent intervals, give an excellent indication of the prognosis, sometimes before any other evidence is available.

2) Much prognostic value lies in the Schilling index and, therefore, prognosis or accurate information as to the progress of such surgical conditions usually cannot be gained from a total leucocyte count and the ordinary differential count which omits the Schilling index.

3) A rising leucocyte count plus a rising Schilling index is indicative of abscess formation, increased infection, or irritation and may be considered unfavorable. However, when they increase together, such can be favorable as long as the Schilling index does not go too high (above 10.0), and if it decreases within two or three days after reaching a peak.

4) A low leucocyte count (below 10,000) with a rising or persistently high Schilling index (8.0 to 10.0) is indicative of inadequate defense and an unfavorable or guarded prognosis.

5) A moderately high leucocyte count (14,000 to 20,000) with a decreasing or low (around 2.0) Schilling index means good resistance and a favorable prognosis.

6) An elevated leucocyte count (between 13,000 and 35,000) which gradually decreases as the Schilling index goes to the right or remains low (around 2.0) is favorable.

7) A low leucocyte count (9,000 to 14,000) with a decreasing Schilling index is favorable; however, if the leucocyte count is too low (below 9,000), the Schilling index will very likely shift to the left, unless it has reached normal limits (below 0.5).

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A Study of Lymphocytic Choriomeningitis Virus

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DURING an investigation of disease in a herd of cows in June, 1940, a number of experimental animals, including 6 guinea pigs, were injected with a brain suspension from one of the dead cows. Within a few days thereafter, one of the guinea pigs sickened. All other experimental animals remained healthy. No bacteria could be detected in the brain of the cow used for injection, and none were found in the tissues of the sick guinea pig when it was killed a short time later. It was found that brain suspensions from this sick guinea pig, when injected by any route, caused other guinea pigs to sicken. Mice were also found to be susceptible. Because no bacteria could be found in the tissues of affected guinea pigs and because material that had passed through Berkefeld V, N, or W filters caused sickness and death in guinea pigs, it was concluded that the active agent was a filterable virus. When guinea pigs or mice were injected intracerebrally, a rather severe meningitis without appreciable encephalitis was produced.

Considering the hosts affected, the symptomatology, and the lesions found on histologic examinations, the virus was tentatively identified as similar to that described by Armstrong and Lillie[1]; Traub[2]; Rivers and Scott[3]; and Findlay, Alcock, and Stern.[4] Hence, comparative studies were made with two known strains of that virus—lymphocytic choriomeningitis. These studies were roughly divided into three parts: (1) animal inoculations, (2) chick embryo propagation, and (3) cross immunity and neutralization tests. Animals were inoculated to learn which were susceptible and also to furnish material for a study of the symptomatology and lesions produced by the unidentified virus for comparison with those caused by known strains. The experimental animals included guinea pigs, rabbits, mice, baby pigs, cattle, young chickens, and rats.

Guinea pigs were the most susceptible, hence they were used for virus propagation. This afforded a great number of these animals for study.

The virulence of the strain seemed to be similar to the "B" strain of lymphocytic choriomeningitis virus reported by Traub[5] before he modified it by serial passage in mice. Death of our guinea pigs occurred after eight to ten days when inoculations were made intracerebrally, and after twelve to fifteen days when inoculations were made subcutaneously. The temperatures rose within twenty-four to forty-eight hours after inoculation, but otherwise the animals appeared healthy until the fifth or sixth day, when they lost appetite and began to lose weight. The temperature remained high until shortly before death. Affected guinea pigs were inactive, remaining in a corner of the cage. Breathing was rapid and shallow. Emaciation was rapid. The posterior parts became weak but there was no paralysis. Catarrhal and purulent conjunctivitis were often noticed. The saliva ran from the mouth, wetting the hair under the lower jaw and down about the front legs. This symptom was more marked than in guinea pigs infected with the virus of equine encephalitis. The virus was found throughout the body, including the blood, brain, spleen, liver, bile, and kidneys. Healthy guinea pigs placed with sick ones remained healthy and were found to be susceptible when tested later. Guinea pigs solidly immune to equine encephalitis were susceptible, as were guinea pigs immune to sporadic bovine encephalomyelitis, McNutt and Waller.[6]

This study was made at the Veterinary Research Institute, Iowa State College, Ames, Iowa.

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Mice were also highly susceptible. Five days after intracranial inoculation, the animals showed a rough hair coat and humped back. They were inactive but ran about the cage when touched. Most of the mice, infected with the strain under study, showed the hind leg and head symptoms described by others for lymphocytic choriomeningitis. When suspended by the tail, the animals exhibited a series of contractions which ended in a tonic extention of the hind legs. The extention of the hind legs and humped back persisted even after death. Several of the mice recovered after a short time, whereas all guinea pigs died. The symptoms observed in rats were very like those seen in guinea pigs but rats often recovered within ten to fifteen days.

Infected baby chicks were most interesting. Twenty-four 10-day-old chicks were inoculated intracerebrally on April 21, 1942, with a liver and spleen suspension from a guinea pig. The chicks remained in apparent good health throughout the experiment and no significant gross lesions were found at any time. On April 28, 3 of the chicks were killed and the virus was found in high concentration in their brains but in only limited amounts in the spleens and livers. On May 11, or twenty days after inoculation, 5 additional chicks were killed. The virus was found in high concentration in both the brain and pooled samples of the livers and spleens of these chicks. On May 20, or twenty-nine days after inoculation, the tissues of 3 more chicks were tested. The virus was found in low concentration in the livers and spleens but none was present in the brain. A still later test, made at about six weeks, showed no virus present in the chick tissues. The baby chicks harbored the virus for four weeks and no doubt it multiplied during that time although apparent symptoms of disease were not noted.

Five baby pigs were inoculated with the strain of virus under study. A 3-day-old pig was injected intracerebrally with 0.3 cc. of a brain, liver, and spleen suspension from an infected guinea pig. Five days later, it showed signs of weakness and inactivity, and died the following day. On postmortem examination, enlarged prescapular lymph glands and an enlarged liver were found. The meninges were slightly congested and, on histologic examination, a slight but definite inflammation was evident. Suspensions of brain, liver, and spleen from this pig were shown to contain the virus in high concentrations. Two 5-day-old pigs were injected intracerebrally with a liver and spleen suspension from an infected guinea pig on Feb. 2, 1942. Nine days later, 1 of these pigs showed tremors and incoördination; at which time it was killed. On postmortem examination, no gross lesions were encountered, but on histologic examination, moderate infiltration of the meninges together with some areas of infiltration of the brain substance were seen. The liver and spleen, but not the brain, of this pig were examined for the virus. It was found in high concentration. On March 2, the second pig was killed while in good health. This pig showed neither virus nor lesions. The virus was instilled into the conjunctival sac of still 2 more pigs. This did not produce apparent disease. Uninoculated control pigs kept with the previously mentioned pigs remained apparently healthy.

Several rabbits inoculated intracerebrally did not show apparent symptoms. Findlay, Alcock, and Stern[4] recovered their virus from rabbit blood shortly after inoculation and demonstrated the presence of neutralizing bodies in the serum.

Because the virus was isolated from a guinea pig injected with tissues from a cow, it seemed desirable to know whether or not cattle could be infected. One 2-year-old Jersey bull was injected subcutaneously with a single massive dose. One 6-year-old pregnant cow was repeatedly injected subcutaneously with moderate amounts of the virus. Two calves were injected intracerebrally and at the same time the virus was instilled into the conjunctival sac. Finally, 2 calves were injected intracerebrally and subcutaneously. Guinea pig tissue virus was employed in all these cattle inoculations. None of the animals showed apparent symptoms. One of the calves was killed thirty days after inocula-

tion. No lesions were observed and no virus was present in its tissues. It appeared very unlikely that cattle were susceptible to the virus. Certainly, no apparent symptoms were produced and the animals were not important carriers of the infection.

All stock guinea pigs that sickened have been examined and no case of lymphocytic choriomeningitis virus infection has been found. In addition, several groups of wild mice about the animal barns have been examined without encountering the lymphocytic choriomeningitis virus.

Aside from the lesions noted in the hogs previously mentioned, the pathologic changes have been studied more extensively in guinea pig. In guinea pigs, the gross lesions were not markedly characteristic. In some cases, the liver was yellowish, hyperemic, and enlarged. The spleen, which appeared normal in a majority of cases, was enlarged in others. Lesions of bronchopneumonia were frequently found but were usually more evident microscopically. When inoculations were made intraperitoneally, a peritoneal exudate was often found. Engorgment of the peripheral vessel of the brain was most noticeable following intracerebral inoculation.

Microscopically, the outstanding lesion consisted of cellular infiltration of the meninges and, to a very moderate extent, of the choroid plexus. The predominant type of cell was the lymphocyte or a lymphocyte-like cell, but mononuclear cells and polymorphonuclear neutrophils were present. Infiltration about the brain vessels was absent or very slight. Cellular infiltration and engorgment of the capillaries were present in the liver. The Kupffer cells were sometimes enlarged and irregular. Areas of consolidation were often present in the lungs and pneumonia of the interstitial type was common.

Rats showed lesions similar to those described for guinea pigs. The lesions in mice were much the same but much more extensive, especially in the brain. The peritoneal cavity contained a large number of lymphocytes.

The disease produced in guinea pigs and mice by the unidentified strain of virus was compared to that produced in the same animals by two known strains of lymphocytic choriomeningitis virus and found to be the same.

Bengston[7] and Tsubaki[8] reported the propagation of the virus of lymphocytic choriomeningitis on the chorioallantoic membrane of developing chick embryos.

In the work to be reported here, the virus isolated from the guinea pig was propagated on the chorioallantoic membrane and also in the yolk sac. The method of Burnet[9] was employed for the membrane inoculations. In the initial inoculations, 0.10 cc. to 0.15 cc. of a 1:10 suspension of infected guinea pig brain in Tyrode's solution was placed on the chorioallantoic membrane of 6- to 7-day-old embryos. After the eighth serial passage, membranes, brain, spleen, and liver of the infected embryos were tested by intracranial inoculations of serial tenfold dilutions into guinea pigs. Dilutions as high as 10^{-4} failed to produce disease in the guinea pigs while guinea pig-brain virus possesses an end point at least 100 times higher than the same weight of embryo tissue of membranes. A titration is shown in table 1.

TABLE 1—Titration of Virus in the Eighth Embryo Passage

Guinea Pig	Emulsion Inoculated	Death	Survival
1	10^{-1}	(11) 1	
2	10^{-2}	(11) 1	
3	10^{-3}	(14) 1	
4	10^{-4}		1
5	10^{-5}		1
6	10^{-6}		1
7	10^{-7}		1
8 2 gp.	10^{-8}		2
9 2 gp.	10^{-9}		2

Figures in parentheses indicate days in which death occurred.

Ordinarily, the inoculation of the virus onto the chorioallantoic membrane caused death of the embryos within a few days but some did not die. A majority of these hatched. Examination of the tissues of some of these hatched chicks showed them to be free of the virus. Because the goal of chick-embryo culturing was to obtain a higher concentration of virus and because it was not successful on the membrane, the yolk sac method was tried. This method was much easier; contaminations are less

frequent and in many instances even better results than with the chorioallantoic membrane are obtained. The method of Cox[10] was employed. Yolk material from one of the eggs which had been inoculated with virus after eight passages on chorioallantoic membrane was chosen as the source of material. Seven to 8-day-old embryos were usually inoculated. When firmly established in the embryos by rapid serial passage, it was found that the virus killed nearly all injected embryos in an average of about five days. After the twentieth passage, tests were made to determine the virus concentration in the embryos. In the first test, 6 embryos which had died five days after inoculation were ground and passed through gauze. This material was weighed and tenfold suspensions were inoculated intracerebrally into guinea pigs, starting with 10^{-1} and extending up to 10^{-9} dilution. A second test was made by grinding each embryo in a small part of its yolk and embryonic fluids, then making the same dilutions and inoculations as in the first test. A third test identical to the first test was again made after the twenty-eighth passage. The results were exactly the same as those shown in table 1 for all three tests. Since the dilutions, the animals inoculated, and the results were the same as in table 1, this table would serve for any of the three tests mentioned previously. With some viruses, the concentration is greater at some period before death of the embryo than at the time of death. Hence, rather extensive but not exhaustive studies were made of infected embryos before death. No higher concentration was found. Because the embryos yielded a lower virus concentration than guinea pig tissues, they were discontinued.

In the cross immunity and neutralization tests that were conducted, the "WE" strain of lymphocytic choriomeningitis virus was employed. It is a standard strain described by Rivers and Scott,[3] who isolated it from the spinal fluid of a human being. Later, the strain showed immunologic similarity to the Armstrong and Traub strains. The strain isolated in Iowa from a guinea pig injected with a tissue suspension from a cow will be designated "T" strain to facilitate explanation of the tests to follow.

Mice immune to either WE or T strains were readily obtained from those that recovered from an initial inoculation of active virus. It was recognized that neutralizing serum could be secured from animals such as the rabbit but it seemed desirable to use guinea pigs for both neutralization tests and immunity tests. Because all guinea pigs died that were injected with active virus, it became necessary to use a vaccine of inactive virus. The method of Traub[11] was first tried but the vaccine did not protect. In a series of trials, it was found that 0.15 per cent formalin was sufficient to inactivate guinea pig tissue virus in twenty-four to thirty-six hours at room temperature. Such a formalized vaccine of 20 per cent guinea pig tissue in physiologic saline was prepared and kept stored at 43 F. Inoculations were made intraperitoneally at weekly intervals. The first dose of vaccine was 1.0 cc.; the second, 2.0 cc.; the third, 4.0 cc.; and the last, 8.0 cc. A week later, a part of the vaccinated guinea pigs were given 500 m.l.d. of active virus by footpad inoculation while the remainder received 100 m.l.d. All those receiving the larger amount died. A majority of those receiving the smaller amount survived, but even these individuals were not totally immune. It was necessary to gradually increase the dose of active virus in order to produce a high immunity. The guinea pigs were so inoculated until they were able to withstand 200,000 m.l.d. A part of these animals, all immunized against the unidentified strain, were then inoculated with the known WE strain in cross immunity tests, while the serum of others was used in virus neutralization tests. A description of a cross immunity test with guinea pigs follows.

Three guinea pigs immune to T strain were inoculated with a brain suspension from a guinea pig infected with WE strain. Guinea pig 1 received 0.10 cc. of a 1:40 suspension by pad inoculation; 2 received the same dose of a 10^{-2} suspension, and 3 were given the same amount of a 10^{-3} suspension. One control, guinea pig 4, a non-

immune, was given 0.10 cc. of the last suspension. On the third day after inoculation, the control guinea pig showed a high temperature which persisted until near death on the twelfth day. The animal showed characteristic symptoms while the immunes remained healthy for a three week period when the experiment was discontinued.

TABLE 2—Guinea Pigs Immune to T Strain, Inoculated with WE Strain by Foot Pad

GUINEA PIG	WE VIRUS SUSPENSION	IMMUNE GUINEA PIG	NONIMMUNE GUINEA PIG
1	1:40	Survived	
2	10^{-2}	Survived	
3	10^{-2}	Survived	
4 (control)	10^{-3}		Died, 12 days

In the neutralization tests, guinea pig brain-virus was employed in 1:10 dilution and multiple dilutions thereof. Equal parts of hyperimmune guinea pig serum were mixed with the virus suspensions and incubated three hours at 37 C. Normal guinea pig serums mixed with the same virus and incubated for three hours at 37 C. were used as controls. Results of a virus neutralization test are shown in table 3. The guinea pigs inoculated with infected-brain and immune serum mixture did not show fever or other symptoms for a period of twenty days when the experiment was concluded. The control guinea pigs which received normal guinea pig-serum and virus mixture showed fever on the fourth day after inoculation and died on the twelfth day.

TABLE 3—Neutralization Test: Serum from Guinea Pig Immune to T Strain Mixed with WE Strain of Lymphocytic Choriomeningitis Virus

GUINEA PIG	WE VIRUS SUSPENSION	IMMUNE GUINEA PIG SERUM	NORMAL GUINEA PIG SERUM
1	10^{-1}	Survived	Not done
2	10^{-2}	Survived	Not done
3 & 5	10^{-3}	Survived	Died
4 & 6	10^{-4}	Survived	Died

When the T strain was substituted for the WE strain as in table 3, the results were identical to those shown in that table.

Mice which had recovered from infection induced by the WE strain were inoculated with the T strain. Likewise, mice recovered from infection with T strain were injected with WE strain. Cross immunity was complete in both cases. A typical test is shown in table 4.

TABLE 4—Mice Recovered from WE Strain Infection, Inoculated with T Strain Guinea Pig-Brain Suspension 1:500, 0.03 cc. to Each Mouse

NUMBER OF MICE REINOCULATED	SURVIVED	DIED
3	3	0
Mice Control 6	0	6 (5-6 days)

DISCUSSION

The fact that the T strain was isolated from tissue of the guinea pig inoculated with material originally taken from a cow does not necessarily mean that the virus came from that source. Experiments demonstrated that exposed cattle showed no symptoms and a trial to recover the virus from an animal killed after inoculation resulted negatively. During the past several years, tissues of several hundred cattle have been examined by methods that would reveal lymphocytic choriomeningitis virus if it had been present. Except for the one instance reported herein, no evidence of the virus was found.

Also, the hypothesis that the virus came from the guinea pig was not proved since this was the only one of several thousand that was found infected. The virus has been reported from North America, England, France, and Japan, hence, it is widespread and the possibility of its origin from guinea pigs is not entirely discounted. Armstrong and Sweet[12] reported the isolation of the virus from grey mice trapped in houses where the virus was isolated from human patients suffering from the infection. Farmer and Janeway[13] reported several cases in which contact of man and grey mice was evident. To test the possibility that grey mice might have carried the virus to the guinea pig, grey mice trapped in the animal barns were examined for the presence of the virus. These examinations were negative, but the per-

centage of carriers is not high and the number of trapped mice was small.

The T strain was more active than either of the two known strains with which it was compared. Mice recovered more easily from infection with either of the two known strains than the T strain. The disease was shorter and more acute in guinea pigs inoculated with the T strain. The serums from guinea pigs hyperimmune to the T strain neutralized both the WE and T strains in the same concentration, in a maximum of 1:10 which contained approximately 10^5 m.l.d. Several months' time was required to satisfactorily immunize guinea pigs. Besides the time required, the dosage of active virus must also be rigorously observed due to the susceptibility of guinea pigs to the virus.

When baby chicks were inoculated with the virus, they exhibited no clinical symptoms of disease over a five week observation period. The virus was found to persist in the tissues of inoculated chicks for four weeks. In such chicks, the virus spread from the point of inoculation to the various other tissues of the body. The ease with which chicks were infected and the long time they carried the virus suggest that they might be a reservoir of infection. In this regard, a limited number of baby chicks from poultry men have been examined for lymphocytic choriomeningitis virus with negative results.

Inoculation of pigs showed slight evidence that swine might be infected under natural conditions; however, the tissues of several hundred pigs picked at random have been examined during the past few years by methods that would reveal the virus, yet all these examinations have been negative.

The many reports in the literature and the unfortunate experience of a number of laboratory workers have shown that lymphocytic choriomeningitis virus is highly dangerous for man.

CONCLUSIONS

1) It is concluded that the "T" strain of virus isolated from a guinea pig, which had been injected with a tissue suspension from a cow, was a strain of lymphocytic choriomeningitis virus. This contention is based on the results of cross immunity tests, neutralization tests, and a study of the disease produced in experimental animals. In all these experiments, known strains of lymphocytic choriomeningitis virus were employed in the comparative studies.

2) It is unlikely that hogs ever show apparent symptoms from natural infection with lymphocytic choriomeningitis virus. It is also unlikely that they serve as carriers.

3) Cattle seem even less susceptible to the virus than do hogs.

4) Although lymphocytic choriomeningitis virus is known to be widespread, it does not appear to be prevalent in Iowa.

5) The experience reported herein serves to put one on his guard against the virus, as so many other reports have done in the past. There are two main dangers: (1) the virus may contaminate other known active agents with which one is working, causing confusing results, and (2) it is a dangerous virus with which to work, hence, may cause accidental infection, even fatal infection, before one is aware of the nature of the virus with which one is working.

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Reaginic Allergy in Cattle

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IN THE PAST little has been reported in the literature on the allergic manifestations in lower animals. Numerous papers have been published on anaphylaxis but the allergic diseases have received little attention save for the studies of serum disease and infectious allergies such as tuberculin and mallein reactions. Among the few scattered reports which do exist are those of Phillips[1] who describes angioneurotic edema; those of Schnelle[2], Pomeroy[3], and Burns[4] who have all studied allergic eczema in dogs; Schroeder[5] who describes a case of cows milk sensitivity in a baby walrus; Bray[6] who, in his textbook, mentions cases of hay fever in purebred cattle, and Vaughn[7] who cites in his book pine pollen sensitivity in a wolfhound. A recent report which made adequate use of diagnostic and therapeutic methods was that of Wittich[8]. He described typical hay fever symptoms in a dog due to ragweed pollen. He showed the typical seasonal occurrence of the symptoms, positive skin, ophthalmic sensitivity, and relief by parenteral administration of ragweed pollen extract. In 1942, Weil and Reddin[9] established experimentally that the immunologic situation of ragweed supersensitivity in cattle is the same as that of man. They described the presence of a sensitizing antibody or reagin in their herd and also described the production of a neutralizing antibody which was thermostabile and was induced by parenteral administration of ragweed antigen. Although the reagin which was found to exist resembled the same type of antibody found in the human subject, all of its characteristics were not studied at that time and the following questions remained unanswered:

1) Is the reagin-antigen combination a true neutralization *i. e.*, irreversible and complete like the reactions of the classical antigens and their antibodies, or is it reversible like the human reagin-antigen combination? (Levine and Coca[10]).

2) Can the reagin of bovine supersensitivity passively sensitize human skin?

3) Can the reagin of human ragweed pollinosis passively sensitize bovine skin?

4) Can dermal sensitivity or reagin production be induced by parenteral administration of ragweed pollen to a nonsensitive cow?

5) Does ophthalmic sensitivity to ragweed pollen exist in cattle?

6) What is the percentage of animals sensitive to ragweed in an average herd?

MATERIAL

In all of the following work, a stock glycerinated extract of giant ragweed, such as is used in human testing, was used and it contained, in the undiluted state, 2.8 mg. of nitro-

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gen per cc. Dilutions of the extract were made with Soerensen's buffered saline solution pH 7.2 so that the test dose of 7, 14, or 28 μg.N was contained in 0.05 cc.

The bovine serum later referred to as ragweed-positive was separated aseptically* from the blood of cow 376 which had previously been found to be strongly sensitive to intracutaneous injection of as little as 7 μg.N. The serum was Berkefeld filtered and preserved with 0.5 per cent phenol.

Human serum was obtained from a patient who had been classified as a class A-B reactor to ragweed when tested the previous fall.*

The animals used in this work were all from our herd used for the production of hyperimmune serum for anthrax and Pasteurella infections. They were purchased from diversified sources and had been in the herd from four months to three years.

METHODS

The *in vivo* methods used for this study were identical with those reported previously.[9] The intracutaneous injections were made into the soft pliable skin over the shoulder with care being taken to keep the sites anterior to the area of transition to the thicker skin of the costal region. To demonstrate the pseudopod formation, the thin skin of the ear is much more satisfactory, but due to the large number of injections which were to be made that area was insufficient. Clear, white skins were used for all of the passive transfer experiments, since readings were more clearly visible in unpigmented skins. Dark skins, however, do not render the reactions less accurate, since they can be readily determined by careful palpation. Whenever possible, the volume injected was limited to 0.05 cc. to avoid mechanical damage from larger quantities. Measurements of the resulting wheal were made fifteen minutes after injection of the test dose, at which time the reaction was at its peak.

Passive transfer of sensitivity was determined by intracutaneous injection of the serum of a sensitive subject into a nonsensitive cow on one day and twenty-four hours later testing the area with the ragweed antigen or experimental mixture.

EXPERIMENTAL

In order to provide a sufficiently large number of negative receptors for the passive sensitization experiments, the entire herd was subjected to intracutaneous test with ragweed antigen. Test doses of 14 and 28 μg. Pollen N were used. The results of some of these tests appear in table 1. From the testing of the 42 animals in our herd, it was found that 40 per cent were sensitive and 60 per cent were not sensitive. Although no effort was made to classify the intensity of response into class A, B, or C, as in human allergy, it will be noted from the table that the same test dose of antigen will elicit entirely different degrees of response in the individual animals when the tests are made in the same region of skin.

TABLE 1—Showing Immediate Response to Intracutaneous Injection of 0.05 cc. Ragweed Pollen Extract

Cow	μG. N IN TEST DOSE	WHEAL (MM.)	Cow	μG. N IN TEST DOSE	WHEAL (MM.)
376	28	30 x 30	446	28	15 x 15
	14	25 x 25		14	8 x 8
	Saline	0		Saline	3 x 3
385	28	18 x 20	449	28	20 x 20
	14	10 x 15		14	18 x 20
	Saline	3 x 3		Saline	5 x 5
440	28	15 x 15	455	28	18 x 20
	14	5 x 8		14	8 x 15
	Saline	0		Saline	0
442	28	15 x 15	463	28	8 x 10
	14	12 x 15		14	5 x 8
	Saline	3 x 3		Saline	3 x 3
444	28	20 x 20	467	28	8 x 8
	14	18 x 20		14	5 x 6
	Saline	5 x 5		Saline	3 x 3

Passive Transfer.—Of the group of negative animals as determined previously, individuals which were quiet and presented a thin pliable skin were selected for the passive transfer experiments. Table 2 shows the results of the passive transfer of sensitivity to 2 nonsensitive cows from a supersensitive animal. The serum of cow 376, which was highly sensitive, was found to sensitize these 2 cows in dilutions as high as 1 : 40. As in man, individuals were found which gave some induration at the control site which had been injected with saline only. Such reactions were found in some cases to be as large as 5 by 5 mm; and our observations of test sites were considered positive only when the wheal formation exceeded markedly this control size.

Quantitative Equivalence of Reagin and Antigen.—In order to study the mutual influence of reagin and antigen in their mixtures, various proportions of antigen and positive serum were mixed and incu-

*The author is indebted to Dr. A. F. Coca for obtaining the human ragweed reaginic serum from one of his patients, and also for conducting the passive transfer experiments to human subjects.

bated overnight at 37 C. The mixtures used in the first phase of this work consisted of a constant amount of antigen extract and varying amounts of positive bovine serum. Three mixtures which were used contained, in mixture 1—equal parts of 1 : 5 serum and undiluted pollen extract; mixture 2—1 : 10 serum and undiluted extract; mixture 3—1 : 15 serum and undiluted extract. Control injections were made of serum alone 1 : 30, 1 : 150 and of buffered saline alone. Twenty-four hours after the intracutaneous injection of these solutions, the sites were all tested with 14 μg.N. The intensity of response diminished as the serum dilution increased. This is shown in table 3. In sites 1 and 2, the antibody dilution was in great excess of the antigen and average size wheals were formed, while in site 3, the antibody was approaching a point of equivalence and a minimum response was noted. The positive serum alone in the control sites, when tested, produced larger wheals than did comparable dilutions of serum in the test mixtures. This specific inhibition of the passively sensitizing properties of the reagin by the antigen has been seen in similar observations in the human subject.[16] The converse of the above experiment is shown in the following test.

TABLE 2—Passive Cutaneous Sensitivity to Ragweed Through Intracutaneous Injection of Serum from Supersensitive Cow 376 into Skin of Nonsensitive Cows 404 and 441

Serum Dilution	1:10	1:20	1:40	1:80	Saline	0	0
Test Dose μg. N 24 hr. later	14	14	14	14	14	14	0.05 cc. Saline
	Diameter of Resulting Wheal in mm.						
Cow 404	20 x 25	20 x 25	5 x 8	3 x 3	pt	pt	pt
Cow 441	25 x 30	20 x 20	8 x 10	3 x 3	pt	pt	pt

pt = Point, slight narrow induration = negative.

In table 3 it was shown that the incubated mixture of antigen and reagin would cause an immediate reaction in the skin of some nonsensitive cows. Since this reaction would invalidate our results in the following test, it was necessary first of all to find a receptor which would not exhibit an immediate reaction to the intracutaneous injection of the mixture used. Cow 469 was selected as such a receptor, and four sites were passively sensitized with 0.05 cc. of ragweed reaginic serum 376. After twenty-four hours these areas were tested with mixture 1 undiluted from our previous test, which represented 70 μg. Pollen N, and a 1 : 10 dilution of the same mixture which contained 7 μg. Pollen N, in the test dose, or 0.05 cc. The two remaining sites, 3 and 4, were tested with dilutions of rag-

TABLE 3—Showing Determination of the Point of Mutual Equivalence (slight antibody excess) in the Quantitative Relations Between Ragweed-Reaginic Bovine Serum 376 and Ragweed Pollen Extract (1 cc. = 2.8 mg.N)

Mixture 1: Ser. 376 1:5 aa Pollen Ext. undil.
Mixture 2: Ser. 376 1:10 aa Pollen Ext. undil.
Mixture 3: Ser. 376 1:15 aa Pollen Ext. undil.
(Mixtures incubated overnight at 37 C.)

Passive sensitization of nonsensitive cow-skin 458 with intracutaneous injections of 0.05 cc. of mixtures.

Site	Mixtures	*Response to test dose of Pollen Ext. given after 24 hr.	Site	Controls	*Response to test dose of Pollen Ext. given after 24 hr.
1	1 IR 15 x 20	10 x 15	4	Serum 376 1 : 30 0.05 cc.	15 x 15
2	2 IR 8 x 8	8 x 8	5	Serum 376 1 : 150	8 x 8
3	3 IR 8 x 8	5 x 5	6	Saline 0.05 cc.	3 x 3

*Test dose of extract 14 μg. N injected intracutaneous; IR = Immediate response mm.

weed pollen extract in equivalent amounts of N. The results of this test are shown in table 4, where it can be seen that the antigen in the dilutions of mixture 1 elicited the same intensity of response as did the control injections of the antigen alone.

TABLE 4—Showing the Failure of Ragweed Reaginic Cow Serum 376 to Inactivate Ragweed Pollen Antigen

Four sites in skin of ragweed-nonsensitive cow 469 received 0.05 cc. ragweed-reaginic serum I.C. After 24 hr., tests were made in these sites with pollen mixture 1 (see table 3) and control solutions.

SITE	MIXTURE 1*	REACTION (MM.)	SITE	CONTROL POLLEN EXT.	REACTION (MM.)
1	Undiluted (70 μg. N)	25 x 28	3	1-2 (70 μg. N)	25 x 25
2	1 : 10 (7 μg. N)	10 x 10	4	1 : 20 (7 μg. N)	10 x 10

*The reagin in this mixture had been shown to be in great excess of the antigen content, which was 70 μg. N in 0.05 cc.

This complete reversibility of union in the antigen and reagin combination is in direct contrast to the observation in the previous experiment.

Passive Transfer of Bovine Reagin to Human Skin.—The results of the attempted passive transfer of the bovine reagin to human skin are thus far inconclusive. Three individuals were selected for this work, 2 of whom were professional receptors having been found to be satisfactory on numerous occasions for passive transfer of sensitivity from other human patients. One of these 2 receptors, Mrs. E. D., experienced, within twenty-four hours, extreme irritation and erythema at the site of the injection of the bovine serum. When these sites were inoculated with a test dose of antigen, a flare-up of this local "serum sickness" took place in the sensitized areas but not in the control area; due to traumatism, and rendered the test useless. The other professional receptor, Mr. C. V., showed only a slightly larger wheal after injection of the test dose in the sensitized area than was evident in the control area. The third receptor, Mr. L. R., had never been used for passive transfer experiments before and consequently nothing was known of his receptivity. Within a few hours he also showed local "serum sickness" at the points of injection of bovine serum and this precluded further use.

Transfer of Human Reagin to Nonsensitive Cow Skin.—The inverse of the foregoing, that is the transfer of human ragweed sensitivity to the cow gave a positive result. Human ragweed reaginic serum was inoculated into the skin of nonsensitive cow 441 which had been found previously to be a good receptor. This serum was from a patient who, on cutaneous test, had previously exhibited a wheal 7/8 in. in diameter, indicating a class A-B reaction. A 1 : 10 dilution of this serum was used intracutaneously and tested the following day by the intracutaneous injection of 14 μg. Pollen N. An immediate reaction of 10 by 10 mm. was evident in comparison with a mere point at the control site. Results of this experiment are shown in table 5. It can readily be seen that even though a heterologous reagin may be transferred to the bovine skin the response is not as great as it would be when transferred to a homologous skin.

TABLE 5—Passive Transfer of Human Ragweed Reaginic Serum MP to Bovine Nonsensitive Skin 441. Serum Given IC;* 24 hr. Later Antigen Test Dose Injected IC

SERUM DILUTION	ANTIGEN TEST DOSE IN N	RESPONSE IN MM.
1 : 10	14 μg.	10 x 10
0.05 cc. Saline	14 μg.	point

*IC = intracutaneously.

Ophthalmic Sensitivity.—Three of the animals which were found to be highly sensitive as determined by our skin tests were tested for ophthalmic reactions to pollen. The pollen was blown onto the eyeball with a small puffer tube. These cows were 376, 446, and 442. Two nonsensitive animals were also tested by pollen administration into the conjunctival sac. Of these 5 cows tested, only 1 (376) showed any reaction. In this case, the left eye, which had received the pollen insufflation, was found to be mildly congested and an increase in

lacrimation was evident. The right eye of these animals was used as control and had received a finely ground powder of sulfanilamide and sulfathiazole. No mechanical irritation was noted from this control powder.

Attempt to Experimently Evoke Reagin Production.—Cow 458 which had been found previously to be nonsensitive to ragweed pollen as determined by intracutaneous test was immunized for five weeks with whole ragweed pollen. Up to the present this animal has not shown any signs of positive skin sensitivity but it has produced neutralizing antibodies. Since the neutralizing antibodies are usually produced, in man at least, only after prolonged treatment, we can then assume that the reagin cannot be produced by injection of the ragweed antigen. This is to be expected inasmuch as this animal, like all of the others, had been potentially exposed for many years to ragweed pollen through inhalation and no reagins had been produced in this animal prior to immunization.

DISCUSSION

The results of the foregoing experiments, taken together with the findings of the previous study with Dr. Weil,[9] make it clear that atopic sensitivity to ragweed pollen in cattle can be frequently demonstrated. The question of the existence of clinical ragweed pollinosis in cattle still remains unanswered in this study. However, the experimental ophthalmic reaction in cow 376 would seem to make this question no longer theoretical but real. In this connection, one should recall the case cited by Bray in England[6] of reputed typical hay fever in purebred cattle. His report is, however, lacking in serologic analysis, the diagnosis having been made on seasonal occurrence of the symptoms. It was not the aim of this study or the previous one to establish in any way the clinical aspect of the disease; and it must be remembered that all of these animals which responded positively to our skin tests had presented no obvious symptoms of pollinosis in the hay fever season.

It therefore remains the duty of the practitioner to make use of these tests in the study of some of the conditions in animals thus far unexplained in an effort to come closer to an accurate diagnosis. Among the conditions which would seem to warrant the earliest study are heaves in horses, eczema, and urticaria of all domestic animals. Following closely in importance are many others which have been listed by Brownlee.[11]

SUMMARY

1) The specific neutralization of the atopic reagin of cattle has been demonstrated.

2) The characteristic failure to inactivate the specific antigen has been demonstrated in the bovine reagin.

3) Passive transfer of human reagin to the skin of cattle was successful. The result of the reverse experiment, cattle reagin to human skin, was inconclusive.

4) The artificial stimulation of reagin production by numerous injections of whole pollen for several weeks failed in one experiment, even though neutralizing antibodies, as in the earlier experiments, were produced in this animal.

5) Ophthalmic sensitivity to ragweed pollen was demonstrated in 1 skin-sensitive cow.

6) In our herd of 40 odd cattle 40 per cent were found skin-sensitive and 60 per cent were negative to test with ragweed pollen extract.

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